Indoleamine 2,3-dioxygenase 1 is highly expressed in glioma stem cells

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ABSTRACT

Recent research has revealed that glioblastoma (GBM) avoids the immune system via strong expression of indoleamine 2,3-dioxygenase 1 (IDO1). IDO1, an enzyme involved in tryptophan metabolism, is now proposed as a new target in GBM treatment, since several reports have demonstrated that IDO1 expression is related to GBM malignancy. On the other hand, it is well known that glioma stem cells (GSCs) are strongly related to the malignancy of GBM. However, there is as yet no report evaluating the relationship between GSCs and IDO1. We therefore examined the expression levels of IDO1 in GSCs in order to identify a new therapeutic target for GBM based on the immune systems of GSCs. In the present study, we employed human GBM cell lines (U-138MG, U-251MG) and patient-derived GSC model cell lines (0125-GSC, 0222-GSC). GSC model cell lines Rev-U-138MG and Rev-U-251MG were established by culturing U-138MG and U-251MG in serum-free media, while differentiated GBM model cell lines 0125-DGC and 0222-DGC were established by culturing 0125-GSC and 0222-GSC in serum-containing media. The expression levels of stem cell markers (Nanog, Nestin, Oct4 and Sox2) and IDO1 protein and mRNA were determined. Rev-U-138MG and Rev-U-251MG formed spheres and their expression levels of stem cell markers were increased as compared to U-138MG and U-251MG. On the other hand, 0125-DGC and 0222-DGC suffered breakdown of sphere formation, despite the original 0125-GSC and 0222-GSC forming spheres, and their expression levels of the markers were decreased. IDO1 expressions were strongly recognized in Rev-U-138MG, Rev-U-251MG, 0125-GSC and 0222-GSC as compared to U-138MG, U-251MG, 0125-DGC and 0222-DGC. These findings demonstrate that GSCs exhibit treatment resistance with immunosuppression via high expression levels of IDO1, and could represent a novel target for GBM treatment.

1. Introduction

Glioblastoma (GBM) is the most common and lethal brain tumor in adults. Despite an established therapy, involving surgical resection followed by radiation therapy and chemotherapy, or integration of novel therapies, such as molecularly targeted agents, angiogenesis inhibitors, etc., clinical outcomes still remain poor. Establishment of a novel therapeutic method is thus needed to improve the prognosis of GBM. Recent research has shown that glioma stem cells (GSCs) contribute to the malignancy of GBM. GSCs are considered to be capable of aberrantly differentiating into diverse cell types, including differentiated glioma cells, in response to their microenvironment [1,2]. Moreover, a previous study has demonstrated that GSCs and differentiated glioma cells could convert between each other [3]. When considering a novel treatment method for GBM, it is important to target not only differentiated glioma cells but also GSCs, and for that reason, a more detailed understanding of the relevant

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featuresisstillneeded.

Recently, the effectiveness of several immunotherapies has been reported to provide a new adjuvant approach for GBM treatment. GBM suppresses autoimmunity via immune checkpoint blockade, such as programmed cell death 1 (PD-1), programmed death-ligand 1 (PD-L1) and cytotoxic T-lymphocyte associated protein 4 (CTLA-4), or enzyme expression such as of indoleamine 2,3-dioxygenase 1 (IDO1) and tryptophan 2,3-dioxygenase 2 [4–6]. Among these factors, IDO1, the rate-limiting enzyme for tryptophan (Trp) metabolism, may be involved in the ability of GBM cells to escape from immune surveillance, and show immunosuppressive function [7–9]. Trp is metabolized to kynurenine by IDO1, and its metabolism results in anergy of effector T cells, while simultaneously fostering maturation and activation of regulatory T cells, and contributing to an immune tolerant environment [10]. Several studies have examined the therapeutic effectiveness of IDO1 inhibitors for cancer, and the possibility has been suggested that IDO1 inhibitors could be used with anticancer drugs [11].

The effectiveness of immunotherapy via IDO1 inhibitors has already been described in a mouse glioma model [12]. Mitsuka et al. reported high expression of IDO1 in human GBM, especially secondary GBM tissue [13], while Hanihara et al. observed a synergistic antitumor effect of IDO1 inhibition with temozolomide (TMZ), a standard chemotherapeutic drug for GBM, in a mouse glioma model [7]. Also, the effectiveness of combined therapy involving radiation therapy with IDO1 inhibitor has been demonstrated in a mouse glioma model [14]. Thus, IDO1 is of interest as a key enzyme for immunotherapy in GBM, although only a few reports have discussed the relation between GSCs and IDO1.

Kesarwani et al. demonstrated that IDO1 accumulates more in GBM than in low-grade glioma [14], but its levels of accumulation in GSCs still remain to be investigated. We hypothesize that IDO1 accumulates more in GSCs rather than differentiated glioma cells, and up-regulations of Trp metabolism due to IDO1 in GSCs could represent a new target for GBM therapy. In the present study, we examined the differences in expression level of IDO1 between GSCs and differentiated glioma cells, while searching for a new therapeutic target for GBM based on the immune systems of GSCs, employing human GBM cell lines.

2. Materials and methods

2.1. Cell culture

The human GBM cell lines U-138MG and U-251MG (purchased from Health Science Research Resources Bank, Sennan, Osaka, Japan) and patient-derived GSC model cell lines 0125-GSC and 0222-GSC were used in the present study. 0125-GSC and 0222-GSC were kindly provided by Dr. Atsushi Natsume and Dr. Toshihiko Wakabayashi, Department of Neurosurgery, Nagoya University School of Medicine (Nagoya, Japan). We examined the detailed features of 0125-GSC and 0222-GSC described previously [15], and applied in several past studies [3,16]. U-138MG and U-251MG were cultured in Dulbecco's modified Eagle's medium (Nissui Pharmaceutical, Tokyo, Japan) containing 10% fetal bovine serum (FBS) (Life Technologies, Grand Island, NY, USA) [17,18]. 0125-GSC and 0222-GSC were cultured in serum-free media, which were composed of serum-free neurobasal media (Invitrogen, Carlsbad, CA, USA) comprising N2 and B27 supplements (Invitrogen), human recombinant basic fibroblast growth factor (bFGF; R&D Systems, Minneapolis, MN, USA), and epidermal growth factor (EGF; R&D Systems).

2.2. Protein expressions

We analyzed the protein expressions of Nanog, Nestin, Oct4, Sox2, and IDO1 in the cell lines by Western blotting (WB). Nanog, Nestin, Oct4 and Sox2 were employed as neural stem cell markers [19–22]. WB was carried out with soluble protein lysates which were prepared from 1×10^7 cells using RIPA buffer (Wako Pure Chemical, Tokyo, Japan) supplemented with protease inhibitor complex mix (Roche Diagnostics, Mannheim, Germany). After determination of the protein contents with a Pierce BCA protein assay kit (Thermo Fisher Scientific, IL, USA), 50 μg proteins were electrophoresed on 12% polyacrylamide gel (TEFCO, Tokyo, Japan). The separated proteins were transferred onto nitrocellulose membranes (GE Healthcare, Tokyo, Japan) for 30 min at 15 V employing Bio-Rad transblot (Bio-Rad Laboratories, Franklin Lakes, NJ, USA). The non-specific binding of the transferred membranes was blocked with 1% skimmed milk dissolved in washing buffer (PBS (-) + 0.1% Tween-20) for 60 min at room temperature. The primary antibodies used for WB were all specific monoclonal antibodies and were treated for 24 h at 4 C. Primary antibodies were purchased from abcam (Tokyo, Japan). Beta-actin was employed as a housekeeping control protein. The secondary antibody was anti-mouse IgG-HRP conjugate (abcam, Tokyo, Japan) and was treated for 60 min at room temperature. The band patterns were analyzed by LAS-4000 (GE Health, Buckinghamshire, UK) after treatment with ECL prime WB detection reagent (GE Healthcare, Buckinghamshire, UK).

2.3. mRNA expressions

We analyzed the mRNA expressions of *Nanog, Nestin, Sox2* and *IDO1* in the cell lines based on the quantitative reverse transcription polymerase chain reaction (RT-PCR). An RNeasy Mini kit (Qiagen Inc., Valencia, CA, USA) was employed for the extraction of mRNA. After determination of the RNA contents with NanoDrop (Thermo Fisher Scientific, IL, USA), the mRNA expression levels were analyzed by the RT-PCR with SYBR-Green Realtime PCR Master Mix (TOYOBO, Osaka, Japan) using a StepOne Real-time PCR System (Applied Biosystems, Foster City, CA, USA). The total RNA was extracted from 1×10^6 cells, and the RT-PCR was carried out with a volume of 20 μl according to the manufacturer's protocol using the SYBR-Green Realtime PCR Master Mix. Amplification was performed by initial denaturing at 90 C for 30 s, reverse transcription at 61 C for 20 min, and a second denaturing at 95 C for 1 min, followed by 45 cycles of extension at 95 C for 15 s, 55 C for 15 s, and 74 C for 45 s. The housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was employed as the control. All primers were synthesized by Operon (Tokyo, Japan). The expression levels of mRNA were calculated employing the following equations by comparing the threshold cycles (CT): $\Delta CT = CT$ of target gene - CT of GAPDH, $\Delta\Delta CT = \Delta CT$ (target cell line) - ΔCT (reference cell line), and ratio $= 2^{-\Delta\Delta CT}$ [23].

2.4. Statistical evaluations

Statistical analyses were performed using the unpaired, Student's t-test. The statistical software IBM SPSS Statistics version 21.0 (International Business Machines Corp., Armonk, NY, USA) was employed for the data analysis, and all quantitative data are presented as the means ± SE. *p*-Values of less than 0.05 were considered as statistically significant.

3. Results

3.1. GBM cell lines acquire stem-like cell features after culture in serum-free media

Initially, we established the GSC model cell lines Rev-U-138MG

and Rev-U-251MG from the human GBM cell lines U-138MG and U-251MG by applying the procedures below. Rev-U-138MG and Rev-U-251MG were established by culturing U-138MG and U-251MG cells in serum-free media for 2 weeks [24]. We had shown previously that a human GBM cell line could regain the features of GSCs in terms of its morphology and up-regulation of CD133 expression when cultured in a serum-free medium [24]. As regards their morphological features, U-138MG and U-251MG cells were scattered and adherent within the serum-containing medium (Fig. 1a and b), whereas Rev-U-138MG and Rev-U-251MG cells formed spheres following culture in serum-free medium (Fig. 1c and d). In contrast, the GSC cell lines 0125-GSC and 0222-GSC originally underwent sphere formation (Fig. 1e and f), but these cells suffered breakdown of sphere formation and became adherent when cultured in serum-containing media (Fig. 1g and h). We designated these cell lines, made by culturing 0125-GSC and 0222-GSC in serum-containing media for 2 weeks, as 0125-DGC and 0222-DGC.

Next, to verify whether these established GSC model cell lines (Rev-U-138MG and Rev-U-251MG) demonstrate stem-like cell features, we examined the protein expressions of neural stem cell markers. Furthermore, to evaluate whether the patient-derived GSC cell lines (0125-GSC and 0222-GSC) lose their stem-like cell features, we again examined the same protein expressions. The expression levels of Nanog, Nestin, Oct4 and Sox2, which are common markers of neural stem cells, in U-138MG, U-251MG, Rev-U-138MG, Rev-U-251MG, 0125-GSC, 0222-GSC, 0125-DGC and 0222-DGC, were determined by WB. The protein expressions of Nanog, Nestin, Oct4 and Sox2 in Rev-U-138MG and Rev−U-251MG were increased as compared to their levels in U-138MG and U-251MG (left half of Fig. 2a). On the other hand, the expressions in 0125-DGC and 0222-DGC were decreased as compared to their levels in 0125-GSC and 0222-GSC (right half of Fig. 2a).

To confirm these results, the mRNA expressions of *Nanog, Nestin,* and *Sox2* in U-251MG, Rev-U-251MG, 0125-GSC and 0125-DGC were measured by using the RT-PCR. The mRNA expression of *Nanog* in Rev-U-251MG increased to 1.23 ± 0.05 fold as compared to the expression in U-251MG ($p < 0.01$; Fig. 2b left). The mRNA expression of *Nestin* in Rev-U-251MG increased to 5.26 ± 0.42 fold as compared to the expression in U-251MG ($p < 0.01$; Fig. 2b center). Also, the mRNA expression of *Sox2* in Rev-U-251MG increased to 5.58 ± 1.50 fold as compared to the expression in U-251MG ($p < 0.01$; Fig. 2b right). On the other hand, the mRNA expression of *Nanog* in 0125-DGC decreased to 0.72 ± 0.01 fold as compared to the expression in 0125-GSC ($p < 0.01$; Fig. 2c left). The mRNA expression of *Nestin* in 0125-DGC decreased to 0.52 ± 0.06 fold as compared to the expression in 0125-GSC ($p < 0.01$; Fig. 2c center). The mRNA expression of *Sox2* in 0125-DGC decreased to 0.19 ± 0.02 fold as compared to the expression in 0125-GSC ($p < 0.01$; Fig. 2c right). These findings confirmed that GSCs and differentiated GBM cells could interconvert between each other.

3.2. GSC cell lines show high expression levels of IDO1

To evaluate the differences in immune system between the GSCs and differentiated glioma cells, the protein expressions of IDO1 in U-138MG, U-251MG, Rev-U-138MG, Rev-U-251MG, 0125-GSC, 0222-GSC, 0125-DGC and 0222-DGC were determined by WB. The protein expressions of IDO1 in Rev-U-138MG and Rev-U-251MG were increased as compared to their levels in U-138MG and U-251MG (left half of Fig. 3a). On the other hand, the protein expressions of IDO1 in 0125-DGC and 0222-DGC were decreased as compared to their levels in 0125-GSC and 0222-GSC (right half of Fig. 3a).

Next, to confirm these results, we examined the mRNA expressions of *IDO1* in U-251MG, Rev-U-251MG, 0125-GSC and 0125-DGC by using the RT-PCR. The mRNA expression of *IDO1* in Rev-U-

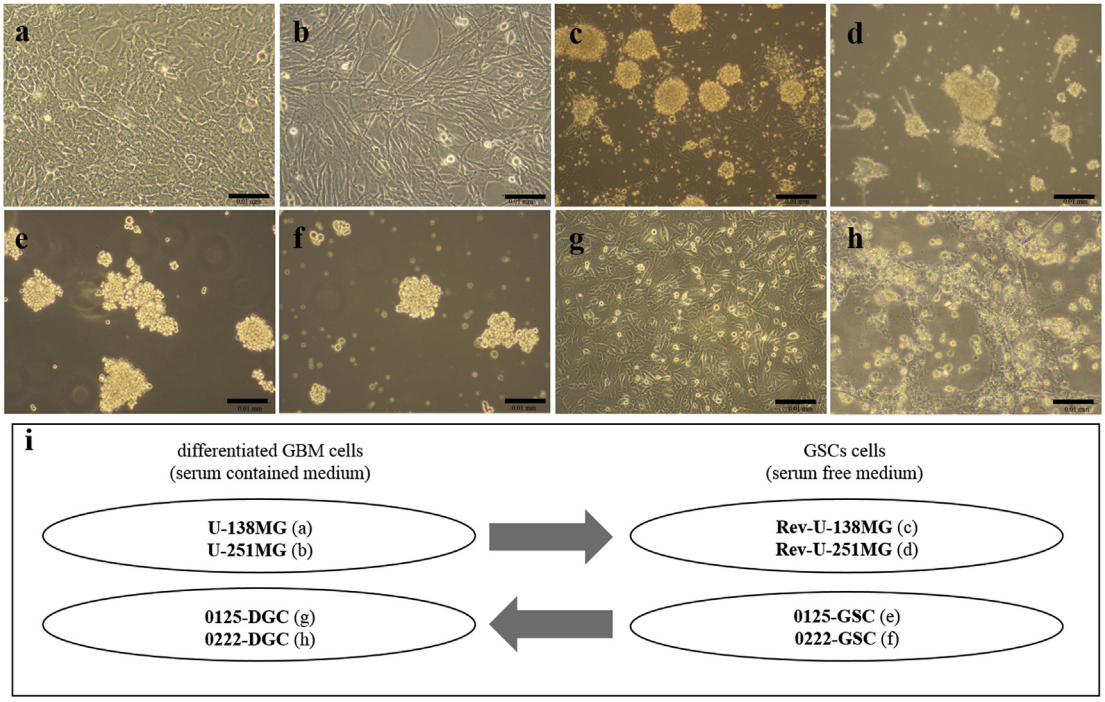

Fig. 1. Comparative morphological changes between glioblastoma cell lines and glioma stem-like cell model cell lines observed under the microscope (x 100). **a-d.** U-138MG (a) and U-251MG (b) were scattered and adherent in the serum-containing medium. Rev-U-138MG (c) and Rev-U-251MG (d), established by culturing U-138MG and U-251MG cells in serum-free media for 2 weeks, formed spheres. Scale bar = 100 μm. **e-h.** Patient-derived glioma stem-like cell model cell lines 0125-GSC (e) and 0222-GSC (f) formed spheres in serum-free media. 0125-DGC (g) and 0222-DGC (h), established by culturing 0125-GSC and 0222-GSC cells in serum-containing media for 2 weeks, suffered breakdown of sphere formation and became adherent. Scale bar = 100 μm. **i.** Schema illustrating the relationship between cell lines and states of cell differentiation.

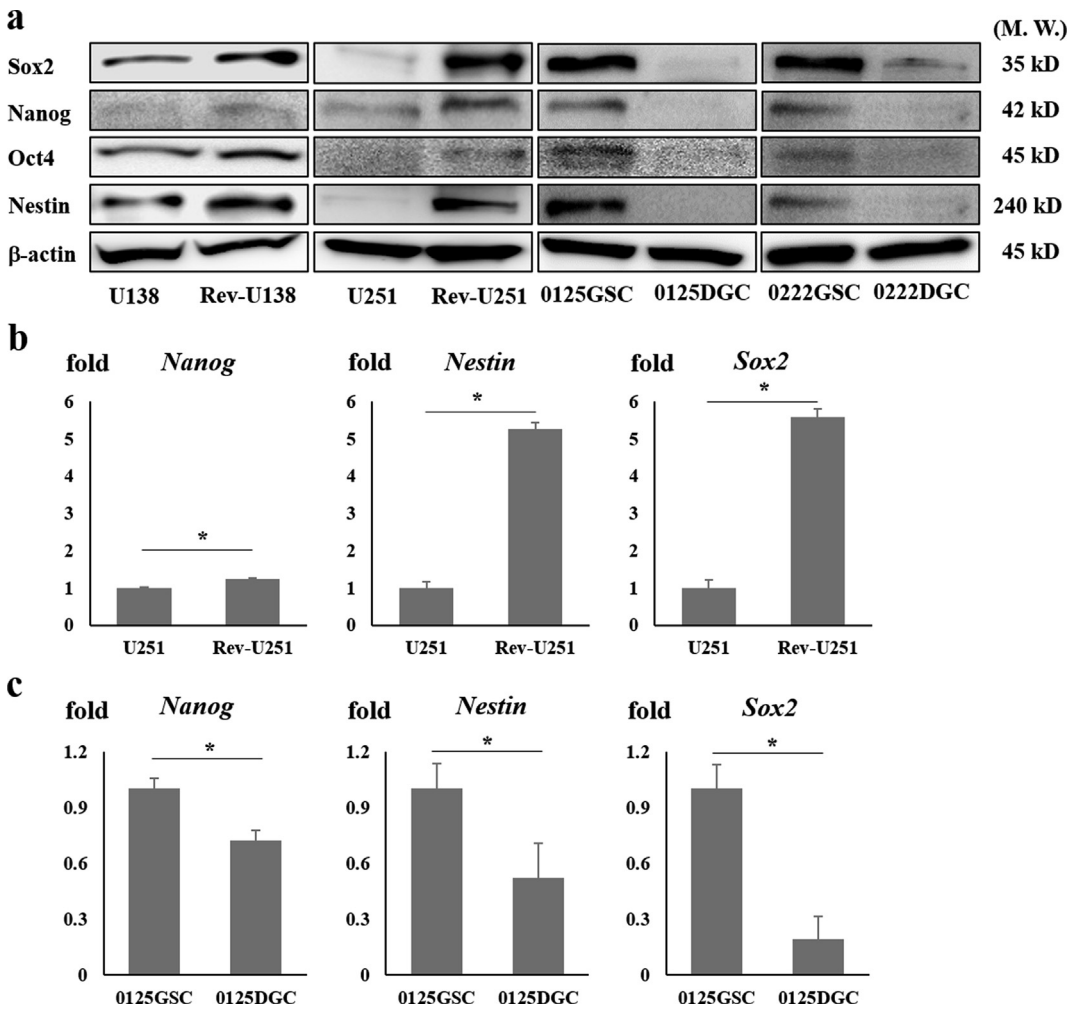

Fig. 2. a. Protein expressions of Nanog, Nestin, Oct4 and Sox2 in U-138MG (U138), Rev-U-138MG (Rev-U138), U-251MG (U251), Rev-U-251MG (Rev-U251), 0125-GSC (0125GSC), 0125-DGC (0125DGC), 0222-GSC (0222GSC) and 0222-DGC (0222DGC) analyzed by Western blotting. **b, c.** mRNA expressions of *Nanog*, *Nestin* and *Sox2* in U251, Rev-U251, 0125GSC and 0125DGC analyzed by the RT-PCR (n = 6). *, $p < 0.01$.

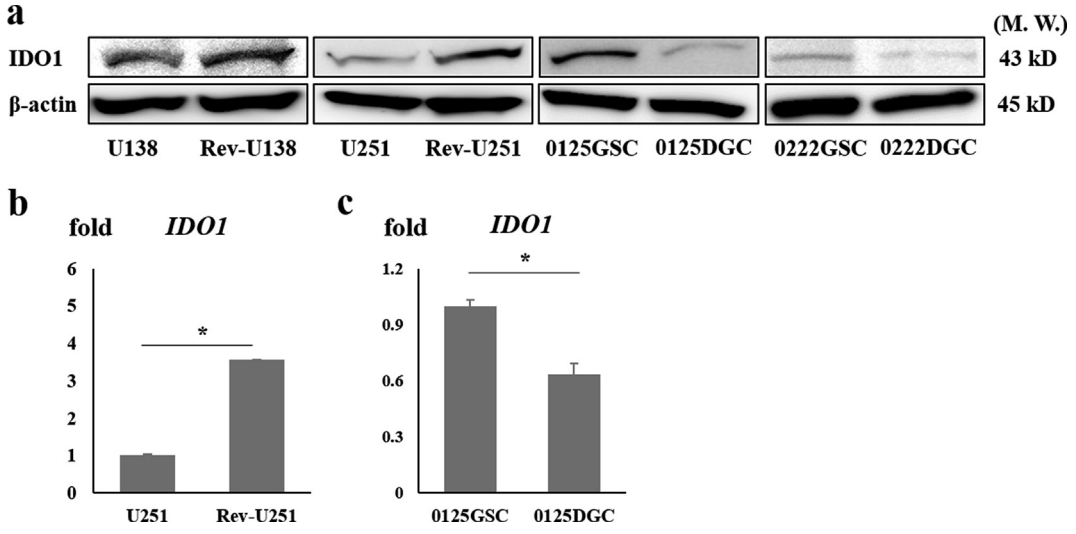

Fig. 3. a. Protein expressions of IDO1 in U138, Rev-U138, U251, Rev-U251, 0125GSC, 0125DGC, 0222GSC and 0222DGC analyzed by Western blotting. **b, c.** mRNA expressions of *IDO1* in U251, Rev-U251, 0125GSC and 0125DGC analyzed by the RT-PCR (n = 6). *, $p < 0.01$.

251MG increased to 3.55 ± 0.09 fold as compared to the expression in U-251MG ($p < 0.01$; Fig. 3b). On the other hand, the mRNA expression of *IDO1* in 0125-DGC decreased to 0.64 ± 0.02 fold as compared to the expression in 0125-GSC ($p < 0.01$; Fig. 3c). These findings suggested that IDO1 expressions are unregulated in GSCs rather than differentiated GBM cells.

3.3. IFN-β suppressed stemness features but stimulates expression levels of IDO1

We demonstrated previously that interferon-beta (IFN-β) could suppress the acquisition of undifferentiated features in GBM cell lines. We therefore attempted to determine whether this IFN-β function could exert suppressive effects on the expression of IDO1 in GSCs. We respectively administered 10 IU/ml of IFN-β (Toray Industries, Tokyo, Japan) to the serum-free medium in which Rev-U-138MG, Rev-U-251MG, GSC-0125 and GSC-0222 were cultured. These cells were then harvested using trypsin-EDTA solution at 4 h. The protein expressions of Nanog, Nestin, Sox2 and IDO1 in the treated and untreated Rev-U138MG, Rev-U-251MG, GSC-0125 and GSC-0222 were determined by WB. As expected, the protein expressions of Nanog, Nestin and Sox2 in treated Rev-U-138MG, Rev-U-251MG, GSC-0125 and GSC-0222 were decreased as compared to the expressions in untreated cell lines. However, the protein expressions of IDO1 in treated Rev-U-138MG, Rev-U-251MG, GSC-0125 and GSC-0222 were increased as compared to the expressions in untreated cell lines (Fig. 4a). Similar results, such as an increased protein expression of IDO1, were also identified in U-251MG and 0125-DGC, when cell of these cell lines were treated with 10 IU/ml of IFN-β (Fig. 4b).

Next, to confirm these results, the mRNA expressions of *Nanog* and *IDO1* in the treated (10 IU/ml IFN-β) Rev-U-251MG and 0125-GSC cells were measured by using the RT-PCR and compared to the levels in the untreated Rev-U-251MG and 0125-GSC cells. The mRNA expression of *Nanog* in treated Rev-U-251MG and 0125-GSC decreased to 0.70 ± 0.02 fold ($p < 0.01$) and 0.84 ± 0.01 fold ($p < 0.01$) as compared to the expression in untreated Rev-U-251MG and 0125-GSC (Fig. 4c). On the other hand, the mRNA expression of *IDO1* in treated Rev-U-251MG and 0125-GSC increased to 13.62 ± 1.88 fold ($p < 0.01$) and 4.47 ± 0.36 fold ($p < 0.01$) as compared to the expression in untreated Rev-U-251MG and 0125-GSC (Fig. 4d). These findings suggest that IFN-β exerts two contrary effects on GBM which involve suppression of stemness features and stimulation of IDO1 expression, and the latter effects exceed the former effect.

4. Discussion

IDO1, the rate-limiting enzyme for Trp metabolism, plays a role in assisting malignant glioma cells to escape from immune surveillance, and causes treatment resistance [7,25]. Moreover, GSCs are considered to be a major factor contributing to the malignancy of GBM. Much research has been carried out on individual GSCs and IDO1, but there is as yet no report on the detailed relation between them. This is the first report to clarify the relationship between GSCs and IDO1.

In the present study, it was demonstrated that the GBM cell lines U-138MG and U-251MG formed spheres with increased levels of

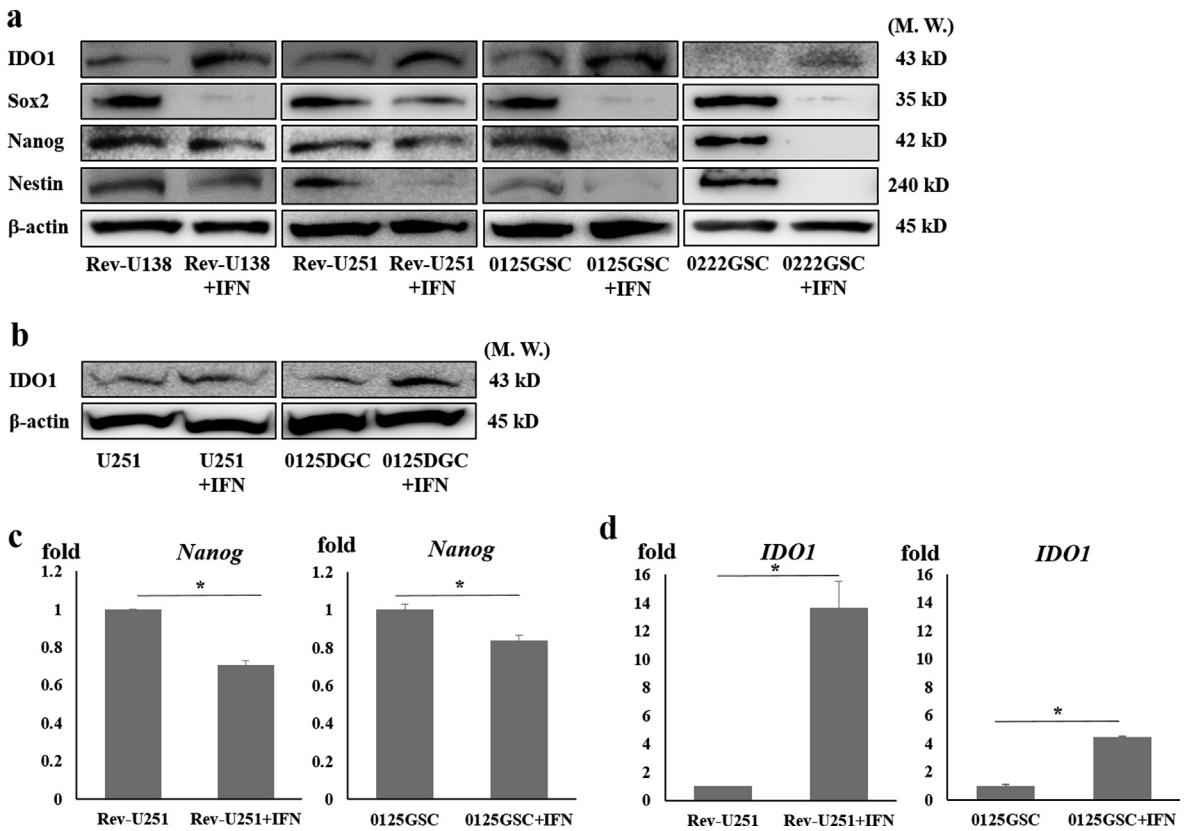

Fig. 4. a. Protein expressions of Nanog, Nestin, Sox2 and IDO1 in Rev-U138, Rev-U251, GSC0125, GSC0222, and these cell lines with IFN-β treatment (Rev-U138+IFN, Rev-U251+IFN, 0125GSC + IFN and 0222GSC + IFN) analyzed by Western blotting. **b.** Protein expressions of IDO1 in U251, 0125DGC, and these cell lines with IFN-β treatment (U251+IFN and 0125DGC + IFN). **c, d.** mRNA expressions of *Nanog* and *IDO1* in Rev-U251, 0125GSC, Rev-U251+IFN and 0125GSC + IFN analyzed by the RT-PCR (n = 6). Rev-U-251MG and 0125GSC cells were treated with 10 IU/ml of IFN-β and harvested after 4 h *, $p < 0.01$.

Nanog, Nestin, and *Oct4* mRNA and Nanog, Nestin, OCT4, and Sox2 protein expression, when cultured in serum-free medium (we designated them as Rev-U-138 and Rev-U-251MG). Also, the expression levels of *IDO1* mRNA and IDO1 protein were increased in Rev-U-138MG and Rev-U-251MG. As described above, we previously found that human GBM cell lines regain the features of GSCs such as sphere formation and up-regulation of CD133 expression when cultured in serum-free medium [24]. Furthermore, Qiang et al. demonstrated that U-251MG expressed the neural stem markers CD133 and Nestin, whereas a lack of neuronal and astrocyte marker microtubule-associated protein 2, β-III tubulin and glial fibrillary acid protein was noted following culture in serum-free medium [19]. On this basis, we regarded Rev-U-138MG and Rev-U-251MG as cell lines of glioma stem-like cells and employed them as materials to evaluate the features of GSCs. We also added the patient-derived GSC cell lines, 0125-GSC and 0222-GSC, to these established GSC model cell lines and examined their expression levels of IDO1 in order to elucidate their immune systems.

Firstly, we clearly confirmed the interconversion between GSCs and normal/differentiated GBM cells, justifying the use of both cell lines. Next, we demonstrated that the expression levels of IDO1, in terms of both mRNA level and protein level, were elevated in the GSC model cell lines as expected. On the other hand, it was confirmed that the expression levels of IDO1 in the patient-derived GSC cell lines were reduced according to the differentiation. Elevation of IDO1 levels in GSCs means that the cells can escape from immune surveillance while producing more IDO1, and this may be strongly related to the malignancy of GBM [7–9]. Such findings are consistent with past research demonstrating that IDO1 rose in malignant gliomas [13,14]. Since IDO1 is highly expressed in malignant gliomas, a novel therapeutic strategy via IDO1 inhibitor has been anticipated for targeting more aggressive and/or high-grade malignant gliomas [14]. Furthermore, since the present study revealed that IDO1 is also highly expressed in GSCs, treatment with IDO1 inhibitor could be additionally expected to serve as a treatment for targeting GSCs, such as in post-operative GBM, because GSCs play an important role in GBM recurrence.

In the present study, we examined the effect of IFN-β which has been used as a drug for glioma treatment. As shown by us previously [24], IFN-β can suppress the acquisition of undifferentiated features in human GBM cell lines. We attempted to determine whether this IFN-β function could exert suppressive effects on the expression of IDO1 in GSCs. In fact, it increased the IDO1 expression in GSCs model cell lines, contrary to expectations. It is well known that interferon-gamma (IFN-γ) can raise the level of IDO1 [14,26–28]. There are also studies demonstrating that IFN-β mediates IDO1, rather than IFN-γ, in astrocytes [29]. Several receptor/ligand signaling pathways upstream to the transcription factors that can regulate IDO1 expression, including IFN-β, have been reported [30]. In the present study, increased levels of IDO1 following IFN-β treatment were found not only in GSC cell lines but also in U-251MG or 0125DGC, and this effect of IFN-β was strongly expressed. If we regard this negative side effect as having appeared more strongly than the expected effect, such results can be considered acceptable. Evidence for the effectiveness of IFN-β administration in GBM therapy has not been reported from phase III randomized control studies. However, there have been many investigations demonstrating an anti-tumor effect of IFN-β or a synergistic anti-tumor effect between TMZ and IFN-β [17,19,31,32]. There is a possibility that we could develop new combination therapies, assuming the fact that IFN-β works in a negative direction for tumor immunotherapy via expression of IDO1. If we could suppress this negative side effect by employing a combination with some immunosuppressive drug, we might expect that the original IFN-β effect could be demonstrated more strongly.

In conclusion, the expression of IDO1, which contributes to treatment resistance in GBM via immunosuppression, was shown to be elevated in GSCs as compared to normal GBM cells. IDO1 is therefore considered to be a valuable and important target when developing a novel effective therapy in GBM.

Declaration of competing interest

We have no potential conflicts of interest to disclosure.

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References

[1] P.B. Gupta, C.L. Chaffer, R.A. Weinberg, Cancer stem cells: mirage or reality? Nat. Med. 15 (2009) 1010–1012.
[2] P.B. Gupta, C.M. Fillmore, G. Jiang, et al., Stochastic state transitions give rise to phenotypic equilibrium in populations of cancer cells, Cell 146 (2011) 633–644.
[3] A. Natsume, M. Ito, K. Katsushima, et al., Chromatin regulator PRC2 is a key regulator of epigenetic plasticity in glioblastoma, Cancer Res. 73 (2013) 4559–4570.
[4] P.E. Fecci, A.B. Heimberger, J.H. Sampson, Immunotherapy for primary brain tumors: no longer a matter of privilege, Clin. Canc. Res. 20 (2014) 5620–5629.
[5] D.M. Pardoll, The blockade of immune checkpoints in cancer immunotherapy, Nat. Rev. Canc. 12 (2012) 252–264.
[6] J. Larkin, V. Chiarion-Sileni, R. Gonzalez, et al., Combined nivolumab and ipilimumab or monotherapy in untreated melanoma, N. Engl. J. Med. 373 (2015) 23–34.
[7] M. Hanihara, T. Kawataki, K. Oh-Oka, et al., Synergistic antitumor effect with indoleamine 2,3-dioxygenase inhibition and temozolomide in a murine glioma model, J. Neurosurg. 124 (2016) 1594–1601.
[8] A.J. Muller, J.B. DuHadaway, P.S. Donover, et al., Inhibition of indoleamine 2,3-dioxygenase, an immunoregulatory target of the cancer suppression gene Bin1, potentiates cancer chemotherapy, Nat. Med. 11 (2005) 312–319.
[9] C. Uyttenhove, L. Pilotte, I. Theate, et al., Evidence for a tumoral immune resistance mechanism based on tryptophan degradation by indoleamine 2,3-dioxygenase, Nat. Med. 9 (2003) 1269–1274.
[10] D.H. Munn, M.D. Sharma, D. Hou, et al., Expression of indoleamine 2,3-dioxygenase by plasmacytoid dendritic cells in tumor-draining lymph nodes, J. Clin. Invest. 114 (2004) 280–290.
[11] T. Miyazaki, K. Moritake, K. Yamada, et al., Indoleamine 2,3-dioxygenase as a new target for malignant glioma therapy: laboratory investigation, J. Neurosurg. 111 (2009) 230–237.
[12] D.A. Wainwright, A.L. Chang, M. Dey, et al., Durable therapeutic efficacy utilizing combinatorial blockade against Ido, CTLA-4, and PD-L1 in mice with brain tumors, Clin. Canc. Res. 20 (2014) 5290–5301.
[13] K. Mitsuka, T. Kawataki, E. Satoh, et al., Expression of indoleamine 2,3-dioxygenase and correlation with pathological malignancy in gliomas, Neurosurgery 72 (2013) 1031–1039.
[14] P. Kesarwani, A. Prabhu, S. Kant, et al., Tryptophan metabolism contributes to radiation- induced immune checkpoint reactivation in glioblastoma, Clin. Canc. Res. 24 (2018) 3632–3643.
[15] S. Yamamuro, Y. Okamoto, E. Sano, et al., Characterization of glioma stem-like cells from human glioblastomas, Int. J. Oncol. 47 (2015) 91–96.
[16] K. Yuki, A. Natsume, H. Yokoyama, et al., Induction of oligodendrogenesis in glioblastoma-initiating cells by IFN-mediated activation of STAT3 signaling, Canc. Lett. 284 (2009) 71–79.
[17] A. Yoshino, A. Ogino, K. Yachi, et al., Effect of IFN-beta on human glioma cell lines with temozolomide resistance, Int. J. Oncol. 35 (2009) 139–148.
[18] A. Yoshino, S. Tashiro, A. Ogino, et al., Gene expression profiles predicting the response to IFN-beta and a combination of temozolomide and IFN-beta in malignant gliomas, Int. J. Oncol. 39 (2011) 529–542.
[19] L. Qiang, Y. Yang, Y.J. Ma, et al., Isolation and characterization of cancer stem like cells in human glioblastoma cell lines, Canc. Lett. 279 (2009) 13–21.
[20] S. Singh, I. Clarke, M. Terasaki, et al., Identification of a cancer stem cell in human brain tumors, Cancer Res. 63 (2003) 5821–5828.
[21] S.Y. Hatano, M. Tada, H. Kimura, et al., Pluripotential competence of cells associated with Nanog activity, Mech. Dev. 122 (2005) 67–79.
[22] K. Mitsui, Y. Tokuzawa, H. Itoh, et al., The homeoprotein Nanog is required for maintenance of pluripotency in mouse epiblast and ES cells, Cell 30 (2003) 631–642.
[23] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method, Methods 25 (2001) 402–408.

[24] S. Yamamuro, E. Sano, Y. Okamoto, et al., Antitumorigenic effect of interferon-beta by inhibition of undifferentiated glioblastoma cells, Int. J. Oncol. 47 (2015) 1647–1654.
[25] J. Godin-Ethier, L.A. Hanafi, C.A. Piccirillo, et al., Indoleamine 2,3-dioxygenase expression in human cancers: clinical and immunologic perspectives, Clin. Canc. Res. 17 (2011) 6985–6991.
[26] J.C. Mbongue, D.A. Nicholas, T.W. Torrez, et al., The role of indoleamine 2,3-dioxygenase in immune suppression and autoimmunity, Vaccines 3 (2015) 703–729.
[27] M. Platten, W. Wick, B.J. Van den Eynde, Tryptophan catabolism in cancer: beyond Ido and tryptophan depletion, Cancer Res. 72 (2012) 5435–5440.
[28] M.W. Taylor, G.S. Feng, Relationship between interferon-gamma, indoleamine 2,3-dioxygenase, and tryptophan catabolism, Faseb. J. 5 (1991) 2516–2522.
[29] H.S. Suh, M.L. Zhao, M. Rivieccio, et al., Astrocyte indoleamine 2,3-dioxygenase is induced by the TLR3 ligand poly(I:C): mechanism of induction and role in antiviral response, J. Virol. 81 (2007) 9838–9850.
[30] W. Li, B.P. Katz, S.M. Spinola, Haemophilus ducreyi lipooligosaccharides induce expression of the immunosuppressive enzyme indoleamine 2,3-dioxygenase via type I interferons and tumor necrosis factor alpha in human dendritic cells, Infect. Immun. 79 (2011) 3338–3347.
[31] A. Natsume, D. Ishii, T. Wakabayashi, et al., IFN-beta down-regulates the expression of DNA repair gene MGMT and sensitizes resistant glioma cells to temozolomide, Cancer Res. 65 (2005) 7573–7579.
[32] J. Park, Y. Joe, T. Kim, et al., Potentiation of antiglioma effect with combined temozolomide and interferon-ß, Oncol. Rep. 16 (2006) 1253–1260.

論文和文要約

表題：Indoleamine 2,3-dioxygenase 1 is highly expressed in glioma stem cells

外科系脳神経外科学専攻　小澤祥成

概要

最近の研究で、膠芽腫は indoleamine 2,3-dioxygenase 1（IDO1）を強く発現することにより、抗腫瘍免疫を回避していることが明らかになった。IDO1 はトリプトファン代謝に関わる酵素であり、IDO1 の発現が膠芽腫の悪性度に関与しているという報告もある。このため IDO1 は膠芽腫の新たな治療標的として注目されている。また、神経膠腫幹細胞も膠芽腫の悪性度と関連していることが示されているが、神経膠腫幹細胞と IDO1 の関係についての報告はまだない。そこで我々は、神経膠腫幹細胞の抗腫瘍免疫システムを標的とした膠芽腫の新たな治療法開発に向けて、神経膠腫幹細胞における IDO1 の発現を検討した。本研究ではヒト膠芽腫細胞株（U-138MG、U-251MG）、及び患者由来の神経膠腫幹細胞モデル細胞株（0125-GSC、0222-GSC）を使用した。また、U-138MG 及び U-251MG を無血清培地で培養することで神経膠腫幹細胞のモデル細胞株 Rev-U-138MG、Rev-U-251MG を新たに樹立した。さらに 0125-GSC 及び 0222-GSC を血清培地で培養することで、分化した膠芽腫細胞株 0125-DGC、0222-DGC を樹立して使用した。ヒト膠芽腫細胞株は無血清培地で培養することで浮遊細胞塊を形成した。反対に、患者由来の神経膠腫幹細胞株を血清培地で培養すると、浮遊細胞塊が解消され接着した。各細胞株について幹細胞マーカー（Nanog、Nestin、sex determining region Y-box2（Sox2）、octamer-binding transcription fancot4（Oct4））、及び IDO1 の蛋白質と messenger RNA（mRNA）の発現を解析したところ、Rev-U-138MG、Rev-U-251MG は U-138MG、U-251MG と比較して幹細胞マーカーを強く発現していた。また、0125-DGC、0222-DGC は 0125-GSC、0222-GSC と比較して幹細胞マーカーの発現が減弱した。さらに、Rev-U-138MG、Rev-U-251MG、0125-GSC、0222-GSC における IDO1 の発現は U-138MG、U-251MG、0125-DGC、0222-DGC と比較して増強していた。これらの結果から、神経膠腫幹細胞は抗腫瘍免疫抑制による治療抵抗性に関与する IDO1 をより強く発現することが明らかとなり、IDO1 が膠芽腫に対する新たな治療標的になり得ることが示唆された。

1. 初めに

膠芽腫は成人における原発性悪性脳腫瘍の中で最も頻度が高く、最も予後の悪い疾患である。膠芽腫に対する治療として、外科的切除および放射線化学療法による後療法が標準治療として行われているが、その予後は極めて悪く、分子標的薬や血管新生阻害薬などを含む様々な新規治療薬の効果が検討されてきたものの、その予後を改善するには至っていない[1, 2, 3]。そのため、膠芽腫に対する新たな治療法を確立することが必要である。近年、がん幹細胞の一種である神経膠腫幹細胞が悪性度の高い膠芽腫により多く存在していることが明らかにされた[4]。膠芽腫は浸潤性の腫瘍であり、根治切除は困難である[5]。そのため、手術後の補助療法が必須となる。しかし、膠芽腫は放射線化学療法に対して抵抗性を示し、その治療抵抗性に神経膠腫幹細胞が関与している。また根治切除が困難であるため、治療後の再発が多く、再発にも神経膠腫幹細胞が関与している[5, 6]。神経膠腫幹細胞は、微小環境に応じて、分化した膠芽腫細胞を含む様々な細胞に分化すると考えられている。さらに神経膠腫幹細胞と膠芽腫細胞は互いに変化しあうことを示した報告もある[7]。膠芽腫に対する新たな治療法を検討するうえでは、膠芽腫細胞だけでなく、神経膠腫幹細胞も対象とすることが重要であり、より詳細な特徴を明らかにする必要がある。

近年、いくつかの免疫療法が膠芽腫の新たな治療法として期待されている。膠芽腫は programmed cell death 1（PD- 1 ）や、programmed death-ligand 1（PD-L1）、cytotoxic T-lymphocyte associated protein 4（CTLA-4）などによる免疫チェックポイントの抑制や、IDO1、tryptophan 2,3-dioxygenase 2 (TDO2)などの酵素を発現することで T 細胞を抑制している[8, 9, 10]。中でも、トリプトファン代謝の律速酵素である IDO1 は、膠芽腫細胞における抗腫瘍免疫の逃避に関与している可能性が示唆されている。トリプトファンは IDO1 によってキヌレニンに代謝され、エフェクターT 細胞の不活性化をもたらし、制御性 T 細胞を成熟、活性化させることで免疫寛容に寄与している[11]。IDO1 阻害剤の抗腫瘍効果を示した報告もなされており、IDO1 阻害薬が抗がん剤として使用される可能性が示唆されている[12]。

IDO1 阻害薬による免疫療法の有効性は既にマウスの膠芽腫モデルで報告されており、膠芽腫において IDO1 が強く発現していることを示した報告もある[13]。また、マウスの膠芽腫モデルで、膠芽腫の標準化学療法薬であるテモゾロミドと IDO1 阻害薬の併用効果を示した報告もなされている[14]。このように IDO1 は膠芽腫の免疫療法を考えるうえで重要な酵素として注目されているが、神経膠腫幹細胞と IDO1 の関係については殆ど調べられていない。

IDO1 は悪性度の低い神経膠腫よりも、悪性度の高い神経膠腫や膠芽腫においてより強く発現していることを示した報告もあるが、神経膠腫幹細胞における IDO1 の発現については明らかになされていない。我々は、神経膠腫幹細胞が、分化した神経膠腫細胞よりも IDO1 を強く発現しており、より強く抗腫瘍免疫を抑制していると考え、本研究において神経膠腫幹細胞と膠芽腫細胞における IDO1 の発現レベルの違いについて検討を行った。

2. 材料と方法

2.1. 細胞培養

ヒト膠芽腫細胞株である U-138MG、U-251MG、及び患者由来の神経膠腫幹細胞株の 0125-GSC、0222-GSC を使用した。0125-GSC、0222-GSC は名古屋大学医学部脳神経外科の夏目敦至先生、及び若林俊彦先生により樹立された患者由来の神経膠腫幹細胞株であり、過去に複数の研究で使用されている[7, 15, 16, 17]。U-138MG、U-251MG は 10%のウシ胎児血清を含むダルベッコ改変イーグル培地（以下、血清培地）で培養した[18]。0125-GSC、0222-GSC の培養には、N2 サプリメント（0.25%）、B27 サプリメント（1%）、ヒト組換え塩基性線維芽細胞成長因子（bFGF; 20 ng/mL）、上皮成長因子（EGF; 20 ng/mL）、L-glutamine（0.5 mM）を加えた無血清の Neurobasal 培地（以下、無血清培地）を使用した[7]。

また、ヒト膠芽腫細胞株である U-138MG、U-251MG を無血清培地で 2 週間培養することで、新たに Rev-U-138MG、Rev-U-251MG を樹立した。さらに、神経膠腫幹細胞株である 0125-GSC、0222-GSC を血清培地で培養することで 0125-DGC、0222-DGC を新たに樹立した。

インターフェロンベータ（IFN-β）添加による実験は、無血清培地に IFN-β を 10 IU/mL 添加した状態で細胞を 7 日間培養した後に行った[7]。

2.2. 蛋白質の発現解析

各細胞株について神経幹細胞マーカー及び IDO1 の蛋白質の発現をウエスタンブロット法（WB 法）で解析した。1×10^7 個の細胞に、蛋白分解酵素阻害剤を添加した RIPA バッファーを加えて蛋白質を回収した。BCA 蛋白アッセイキットで蛋白濃度を測定した後に、50 μg の蛋白質を 12%ポリアクリルアミドゲルで電気泳動した。分離した蛋白質は Bio-Rad トランスブロットを用いて 15 V、30 分間でニトロセルロース膜に転写した。転写された膜の非特異的な結合は洗浄緩衝液に 1%のスキムミルクを加え、室温で 60 分

間ブロックした。一次抗体は神経幹細胞マーカーの、Nanog、Nestin、Oct4、Sox2 を使用し[19, 20, 21]、ハウスキーピング蛋白として β アクチンを使用した。一次抗体はすべて特異的モノクローナル抗体により、4℃で 24 時間処理した。二次抗体は抗マウス IgG-HRP 抗体を使用し、室温で 60 分間処理した。バンドパターンは ECL prime WB detection reagent で処理した後 LAS-4000 で解析した。

2.3. mRNA の発現解析

各細胞株について *Nanog*、*Nestin*、*Sox2*、及び *IDO1* の mRNA の発現を reverse transcription polymerase chain reaction 法 （RT-PCR 法）により解析した。1×10^6 個の細胞から RNeasy MINI kit を用いて mRNA を抽出し、NanoDrop で RNA 量を測定した後に、StepOne Real-time PCR System を用いて解析した。SYBR-Green Realtime PCR Master Mix を加えて 20 μL とし、増幅は 45 サイクル行った。コントロールにはハウスキーピング遺伝子である *GAPDH* を用いた。プライマーは過去の報告で使用された配列を参考にして作成した[7, 22, 23]。mRNA の発現量は ΔΔCT 法を用いて算出した。

2.4. 神経膠腫幹細胞の IFN-β 添加による IDO1 の発現解析

神経膠腫幹細胞である Rev-U-138MG、Rev-U-251MG、GSC-0125、GSC-0222 に対して通常の無血清培地に IFN-β を添加し、IFN-β で処理した細胞群と、未処理の細胞群で Nanog、Nestin、Sox2 と IDO1 の蛋白発現を WB 法で評価した。また Rev-U-251MG、0125-GSC において同様に無血清培地に IFN-β を添加し、*Nanog*、*IDO1* の mRNA の発現を RT-PCR 法で測定した。

2.5. 統計解析

統計は Student‘s t-test を用いた。統計ソフトには IBM SPSS Statistics version 21.0 を使用し、全てのデータは平均値と標準誤差で示した。p 値が 0.05 未満の場合を統計学的に有意とみなした。

3. 結果

3.1. 無血清条件下での細胞分化の評価

我々は過去に、膠芽腫細胞株を無血清培地で一定期間継続して培養すると、膠芽腫細胞株が幹細胞マーカーである CD133 を高発現することを報告している[7]。膠芽腫細胞株である U-138MG、U-251MG は接着細胞であるが、無血清培地で培養した Rev-U-138MG、Rev-U-251MG は浮遊細胞塊を形成した。反対に、0125-GSC、0222-GSC は浮遊細胞塊を形成していたが、血清培地で培養することで浮遊細胞塊は解消され、接着した。

次に、各細胞株において、神経幹細胞マーカーである Nanog、Nestin、Oct4、Sox2 の蛋白の発現量を WB 法で測定し、分化度の評価を行った。無血清培地で樹立した Rev-U-138MG、Rev-U-251MG は元の U-138MG、U-251MG と比べ、Nanog、Nestin、Oct4、Sox2 の発現が増強していた。また、血清培地で樹立した 0125-DGC、0222-DGC に関しては、0125-GSC、0222-GSC と比較して Nanog、Nestin、Oct4、Sox2 の発現が減弱した。

これらの結果をさらに確認するため U-251MG、Rev-U-251MG、0125-GSC、0125-DGC において *Nanog*、*Nestin*、*Sox2* の mRNA の発現を RT-PCR 法で解析した。結果として、蛋白発現と同様に、Rev-U-251MG では U-251MG と比較して *Nanog*、*Nestin*、*Sox2* の mRNA の発現が増強しており、また、0125-DGC でも同様に 0125-GSC と比較して *Nanog*、*Nestin*、*Sox2* の mRNA の発現が減弱していた。これらの結果から、神経膠腫幹細胞と分化した膠芽腫細胞は相互に変換し得ること、及び分化した膠芽腫細胞は無血清培地で培養することで神経膠腫幹細胞の特徴を （再)獲得し得ることが確認された。

3.2. 神経膠腫幹細胞における IDO1 の発現解析

続いて、神経膠腫幹細胞と分化した膠芽腫細胞における IDO1 の発現を解析した。U-138MG、U-251MG、Rev-U-138MG、Rev-U-251MG 及び 0125-GSC、0222-GSC、0125-DGC、0222-DGC の IDO1 の蛋白発現を WB 法で評価したところ、Rev-U-138MG、Rev-U-251MG では U-138MG、U-251MG と比較して IDO1 の発現が増強していた。また同様に 0125-DGC、0222-DGC では 0125-GSC、0222-GSC と比較して IDO1 の発現が減弱していた。

次に U-251MG、Rev-U-251MG 及び 0125-GSC、0125-DGC における *IDO1* の mRNA の発現を RT-PCR 法を用いて解析した。結果は同様に、Rev-U-251MG では U-251MG と比較して *IDO1* の発現が増強しており、0125-DGC では 0125-GSC と比較して *IDO1* の発現が減弱していた。これらの結果から、神経膠腫幹細胞は分化した膠芽腫細胞と比較して IDO1 を強く発現していることが示された。

3.3. IFN-β 添加による膠芽腫細胞の分化、及び IDO1 発現の変化

我々は過去に、IFN-β が神経膠腫細胞の脱分化の過程を抑制することを示してきた[7]。そこで、IFN-β が膠芽腫細胞の脱分化を抑制することで IDO1 の発現を抑制し得るかを検討した。IFN-β で処理した細胞群と、未処理の細胞群で Nanog、Nestin、Sox2 と IDO1 の蛋白発現を WB 法で評価した。結果として、IFN-β 処理群では未処理群と比較して、Nanog、Nestin、Sox2 の発現は減少したものの、IFN-β 処理群では未処理群と比較して IDO1 の蛋白の発現が増強していた。また、U-251MG、0125-DGC において、血清培地に IFN-β を 10 IU/mL 添加し、IFN-β で処理した細胞群と、未処理の細胞群で IDO1 の蛋白の発現を WB 法で評価したところ、同様に IFN-β で処理した群は未処理の群と比較して IDO1 の発現が増強していた。

次に、Rev-U-251MG、0125-GSC において同様に IFN-β を添加し、*Nanog*、*IDO1* の mRNA の発現を RT-PCR 法で測定した。結果として、IFN-β 処理群では未処理群と比較して *Nanog* の発現は減弱していたが、*IDO1* の発現は増強していた。これらの結果から、IFN-β は膠芽腫に対して、幹細胞性質獲得の抑制、及び IDO1 の発現の促進という二つの相反する効果を示し、後者が前者を上回ることが示唆された。

4. 考察

トリプトファン代謝における律速酵素である IDO1 は、悪性神経膠腫において抗腫瘍免疫の逃避に関与しており、治療抵抗性の原因となっている[12]。さらに神経膠腫幹細胞は膠芽腫の悪性度の大きな要因となっている[13]。神経膠腫幹細胞や IDO1 については多くの研究がなされているが、両者の詳細な関係については未だ報告されていない。本研究は、神経膠腫幹細胞において IDO1 が強く発現していることを明らかにした初めての報告である。

膠芽腫細胞株である U-138MG、U-251MG を無血清培地で一定期間培養することで樹立した Rev-U-138MG、Rev-U-251MG は、浮遊細胞塊を形成し、幹細胞マーカーである Nanog、Nestin、Sox2、Oct4 を強く発現していた。我々は過去に、膠芽腫細胞株を無血清培地で培養することで、浮遊細胞塊の形成や、CD133 を高発現することを報告している[7]。また、U-251MG は無血清培地で培養することで神経幹細胞マーカーである CD133、Nestin の発現が増強し、神経細胞やアストロサイトのマーカーである microtubule-associated protein 2（MAP2）、tubulin beta Ⅲ（TUBB3）、glial fibrillary acidic protein（GFAP）の発現がみられなくなることを示した報告もある[24]。このことから Rev-U-138MG、Rev-U-251MG を神経膠腫幹細胞とみなし、また患者由来の神経膠腫幹細胞株である 0125-GSC、0222-GSC を用いて IDO1 の発現量を調べた。

まず、神経膠腫幹細胞と分化した膠芽腫細胞が相互に変換し得ることを確認した。次に神経膠腫幹細胞は元の分化した膠芽腫細胞と比較して、IDO1 の蛋白質、及び mRNA の発現が増強していた。一方で、患者由来の神経膠腫幹細胞は、分化することで、IDO1 の発現が減少した。これらの結果から、神経

膠腫幹細胞は IDO1 を強く発現していることが明らかになった。また、神経膠腫幹細胞は IDO1 を強く発現することで、腫瘍免疫を抑制しており、このことが膠芽腫の治療抵抗性に強く関与していることが示唆された。IDO1 の発現は神経膠腫の悪性度に比例して増強することを示した報告もあり、膠芽腫を含む悪性の高い神経膠腫並びに神経膠腫幹細胞において強く発現している IDO1 は、膠芽腫治療を考えるうえで有望な治療標的になり得ると考えられた[4]。さらに、神経膠腫幹細胞は膠芽腫の再発にも寄与していることが知られており、IDO1 を標的とする治療は、膠芽腫の再発予防にも有効となり得ると考えられた。

IFN-β は腫瘍増殖抑制、アポトーシス誘導や、p53 を介した O6-methylguanine-DNA methyltransferase（MGMT）の遺伝子発現を抑制してテモゾロミドに対する薬剤感受性を増強するなど、膠芽腫に対する抗腫瘍効果が多数報告された薬剤である[25.26]。我々は過去に、IFN-β が神経膠腫細胞の脱分化の過程を抑制することを報告している[7]。そこで、本研究において IFN-β が神経膠腫幹細胞における IDO1 の発現を抑制し得るかを検討したが、予想に反し、IFN-β 処理群では未処理群と比較して IDO1 の発現が増強していた。IFN-γ が IDO1 の発現を増強することはよく知られており、中には IFN-β にも同様の効果があるとした報告もある[27.28.29.30.31.32]。IFN-β が IFN-stimulated gene 3 並びに IFN-stimulated response element を介して IDO1 の発現を増強することが知られており、本研究では、IFN-β の IDO1 を発現させる効果が、脱分化の過程を抑制する効果よりも強く現れた可能性が考えられる。膠芽腫細胞に対する IFN-β の抗腫瘍効果、あるいはテモゾロミドとの併用効果については、基礎実験レベルで有効性を示した報告が複数なされている[25.26.33.34]。これらの報告を受け、IFN-β の膠芽腫に対する有効性が第Ⅲ相臨床試験において検証されたが、生存期間を延長することはできなかった。その原因の一つに、IFN-β により IDO1 の発現が増強したことが関与していると考えれば、IFN-β と IDO1 の作用を抑制する薬剤を併用することにより、IFN-β の抗腫瘍効果を得ることができる可能性が考えられた。

腫瘍免疫抑制を介して膠芽腫の治療抵抗性に寄与していると考えられている IDO1 は、神経膠腫幹細胞において、分化した膠芽腫細胞よりも強く発現していることが示された。この結果より、IDO1 は膠芽腫に対する新たな治療標的になり得ることが示唆された。

参考文献

[1] Sandmann T, Bourgon R, Garcia J, et al. Patients With Proneural Glioblastoma May Derive Overall Survival Benefit From the Addition of Bevacizumab to First-Line Radiotherapy and Temozolomide: Retrospective Analysis of the AVAglio Trial. J Clin Oncol 33: 2735-44, 2015

[2] McGirt MJ, Than KD, Weingart JD, et al. Gliadel (BCNU) wafer plus concomitant temozolomide therapy after primary resection of glioblastoma multiforme. J Neurosurg 3: 583-8, 2009

[3] Stupp R, Taillibert S, Kanner A, et al. Maintenance Therapy With Tumor-Treating Fields Plus Temozolomide vs Temozolomide Alone for Glioblastoma: A Randomized Clinical Trial. JAMA 23: 2535-43, 2015

[4] Mitsuka K, Kawataki T, Satoh E, et al. Expression of indoleamine 2,3-dioxygenase and correlation with pathological malignancy in gliomas. Neurosurgery 72: 1031-9, 2013

[5] Gupta PB, Chaffer CL, Weinberg RA. Cancer stem cells: mirage or reality? Nat Med 15: 1010-2,2009

[6] Gupta PB, Fillmore CM, Jiang G, et al. Stochastic state transitions give rise to phenotypic equilibrium in populations of cancer cells. Cell 146: 633-44, 2011

[7] Yamamuro S, Sano E, Okamoto Y, et al. Antitumorigenic effect of interferon-β by inhibition of undifferentiated

glioblastoma cells. Int J Oncol 47: 1647-54, 2015
[8] Fecci PE, Heimberger AB, Sampson JH, et al. Immunotherapy for primary brain tumors: no longer a matter of privilege. Clin Cancer Res 20: 5620-9, 2014
[9] Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer 12: 252–64, 2012
[10] Larkin J, Sileni VC, Gonzalez R et al. Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma. N Engl J Med 373: 23–34, 2015
[11] Munn DH, Sharma MD, Hou D, et al. Expression of indoleamine 2,3-dioxygenase by plasmacytoid dendritic cells in tumor-draining lymph nodes. J Clin Invest 114: 280–290, 2004
[12] Wainwright DA, Chang AL, Dey M, et al. Durable therapeutic efficacy utilizing combinatorial blockade against IDO, CTLA-4, and PD-L1 in mice with brain tumors. Clin Cancer Res 20: 5290-301, 2014
[13] Kesarwani P, Prabhu A, Kant S, et al. Tryptophan Metabolism Contributes to Radiation-Induced Immune Checkpoint Reactivation in Glioblastoma. Clin Cancer Res 24: 3632-43, 2018
[14] Hanihara M, Kawatani T, Oh-Oka K, et al. Synergistic antitumor effect with indoleamine 2,3-dioxygenase inhibition and temozolomide in a murine glioma model. J Neurosurg 124: 1594-601, 2016
[15] Natsume A, Ito M, Katsushima K, et al. Chromatin regulator PRC2 is a key regulator of epigenetic plasticity in glioblastoma. Cancer Res. 73: 4559-70, 2013
[16] Yamamuro S, Okamoto Y, Sano E, et al. Characterization of glioma stem-like cells from human glioblastomas. Int J Oncol. 47: 91-96, 2015
[17] Yuki K, Natsume A, Yokoyama H, et al. Induction of oligodendrogenesis in glioblastoma-initiating cells by IFN-mediated activation of STAT3 signaling. Cancer Lett 284: 71-9, 2009
[18] Yoshino A, Ogino A, Yachi K, et al, Effect of IFN-beta on human glioma cell lines with temozolomide resistance. Int. J. Oncol 35: 139-148, 2009
[19] Lathia, Mack SC, Mulkearns-Hubert EE, et al. Cancer stem cells in glioblastoma. Genea Dev 29:1203-17, 2015
[20] Singh S, Clarke ID, Terasaki M, et al. Identification of a cancer stem cell in human brain tumors. Cancer Res 63: 5821-5828, 2003
[21] Schaijik B, Davis PF, Wickremesekera AC, et al. Subcellular localisation of the stem cell markers OCT4, SOX2, NANOG, KLF4 and c-MYC in cancer: a review. J Clin Pathol 71: 88-91, 2018
[22] Koike T, Wakabayashi T, Mori T, et al. Sox2 promotes survival of satellite glial cells in vitro. BBRC 14: 269-74, 2015
[23] Sueun L, Miyoung Y, Jinwook K, et al. Nestin expression and glial response in the hippocampus of mice after trimethyltin treatment. Acta Histochem 116: 1276-88, 2014
[24] L Qiang, Yong Y, Yan-jun M, et al. Isolation and characterization of cancer stem like cells in human glioblastoma cell lines. Canc. Lett 279: 13-21, 2009
[25] Yoshino A, Tashiro S, Ogino A, et al, Gene expression profiles predicting the response to IFN-β and a combination of temozolomide and IFN-β in malignant gliomas. Int. J. Oncol 39: 529-42, 2011
[26] Natsume A, Ishii D, Wakabayashi T, et al, IFN-beta down-regulates the expression of DNA repair gene MGMT and sensitizes resistant glioma cells to temozolomide. Cancer Res 65: 7573-9, 2005
[27] Adams O, Besken K, Oberdorfer C, et al. Role of indoleamine-2,3-dioxygenase in alpha/beta and gamma

interferon-mediated antiviral effects against herpes simplex virus infections. J Virol 78: 2632-2636, 2004
[28] Mbongue JC,Nicholas DA, Torrez TW, et al. The Role of Indoleamine 2, 3-Dioxygenase in Immune Suppression and Autoimmunity. Vaccines 3: 703-729, 2015
[29] Platten M, Wick W, Eynde B, et al. Tryptophan catabolism in cancer: beyond IDO and tryptophan depletion. Cancer Res 72: 5435-5440, 2012
[30] Su J, Chen X, Huang Y, et al. Phylogenetic distinction of iNOS and IDO function in mesenchymal stem cell-mediated immunosuppression in mammalian species. Cell Death Differ 21: 388-396, 2014
[31] Suh HS,Zhao ML, Rivieccio M, et al. Astrocyte indoleamine 2,3-dioxygenase is induced by the TLR3 ligand poly(I:C): mechanism of induction and role in antiviral response. J Virol 81: 9838-9850, 2007
[32] Li W,Katz BP, Spinola SM. Haemophilus ducreyi lipooligosaccharides induce expression of the immunosuppressive enzyme indoleamine 2,3-dioxygenase via type I interferons and tumor necrosis factor alpha in human dendritic cells. Immun 79: 3338-3347, 2011
[33] Park J, Joe YA, Kim TG, et al. Potentiation of antiglioma effect with combined temozolomide and interferon-beta. Oncol Rep 16: 1253-1260, 2006
[34] Wakabayashi T, Kayama T, Nishikawa R, et al. A multicenter phase I trial of combination therapy with interferon-β and temozolomide for high-grade gliomas (INTEGRA study): the final report. J Neurooncol 104:573-577, 2011