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The expression pattern of VISTA in the PBMCs of relapsing-remitting multiple sclerosis patients: A single-cell RNA sequencing-based study

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ABSTRACT

Multiple sclerosis (MS) is an inflammatory disease of the central nervous system (CNS). Dysregulated immune responses have been implicated in MS development. Growing evidence has indicated that inhibitory immune checkpoint molecules can substantially regulate immune responses and maintain immune tolerance. V-domain Ig suppressor of T cell activation (VISTA) is a novel inhibitory immune checkpoint molecule that can suppress immune responses; however, its expression pattern in the peripheral blood mononuclear cells (PBMCs) of relapsing-remitting multiple sclerosis (RRMS) has not thoroughly been studied. Herein, we evaluated *Vsir* expression in PBMCs of RRMS patients and characterized the expression pattern of the *Vsir* in the PBMCs of MS patients. Besides, we investigated the effect of fingolimod, IFNβ-1α, glatiramer acetate (GA), and dimethyl fumarate (DMF) on *Vsir* expression in PBMCs of RRMS patients. Our results have shown that *Vsir* expression is significantly downregulated in the PBMCs of patients with RRMS. Besides, the single-cell RNA sequencing results have demonstrated that *Vsir* expression is downregulated in classical monocyte, intermediate monocytes, non-classical monocytes, myeloid DCs (mDC), Plasmacytoid dendritic cells (pDCs), and naive B-cells of PBMCs of MS patients compared to the control. In addition, DMF, IFNβ-1α, and GA have significantly upregulated *Vsir* expression in the PBMCs of RRMS patients. Collectively, the current study has shed light on *Vsir* expression in the

Abbreviations: MS, Multiple sclerosis; CNS, central nervous system; VISTA, V-domain Ig suppressor of T-cell activation; VSIR, V-Set Immunoregulatory Receptor; PBMCs, peripheral blood mononuclear cells; RRMS, relapsing-remitting multiple sclerosis; IFNβ-1α, interferon-beta 1-alpha; GA, glatiramer acetate; DMF, dimethyl fumarate; PD-1, Programmed cell death protein 1; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; CSF, Cerebrospinal fluid; GEO, Gene Expression Omnibus; PCA, principal component analysis; HVG, highly variable genes; DE, differential expression; UMAP, uniform manifold approximation; t-SNE, t-distributed stochastic neighbor embedding; cDNA, complementary DNA; RT-PCR, Real-time polymerase chain reaction; GAPDH, Glyceraldehyde-3-Phosphate Dehydrogenase; TIM-3, T cell immunoglobulin and mucin-domain expression containing-3; EAE, experimental autoimmune encephalomyelitis; SLE, systemic lupus erythematosus; DMTs, disease-modifying treatments; IL-9, interleukin 9; FDA, The Food and Drug Administration.

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PBMCs of MS patients; however, further studies are needed to elucidate the significance of VISTA in the mentioned immune cells.

1. Introduction

MS is a severe autoimmune condition that affects approximately 2.5 million people worldwide [1]. Although its precise pathogenesis remains unknown, it is believed to be a multifactorial disease influenced by genetic and environmental factors [2]. Recent advances in single-cell RNA sequencing have already started to prepare remarkable molecular insights into the brain's complexity by discovering novel cellular subtypes [3]. Despite many challenges, this approach has also provided ample opportunities to study the immune cells in various conditions [4].

Growing evidence has indicated that immune checkpoint molecules can substantially regulate immune responses [5]. VISTA, also known as *Vsir*, is a cell surface inhibitory molecule that can be expressed in myeloid and lymphoid cells [6,7]. VISTA can be distinguished from programmed cell death 1 (PD-1) and cytotoxic T lymphocyte-associated antigen (CTLA-4) in that it may act as both a ligand (by binding the co-inhibitory receptor P-selectin glycoprotein ligand-1 in acidic conditions) and a receptor (by attaching the ligand V-set and immunoglobulin domain-containing protein 3) [8]. It has been shown that neutralizing VISTA can improve T cell activation and decrease the development of forkhead box P3 $(FOXP3)^{+}CD4^{+}$ regulatory T cells [9]. Besides, it has been demonstrated that *Vsir* knockout can impair peripheral tolerance and lead to multiorgan chronic inflammation development. Indeed, *Vsir* knockout can lead to the development of hyperresponsive $CD4^{+}$ and $CD8^{+}$ T cells [10]. Despite the regulatory effect of VISTA on immune responses, *Vsir* expression pattern and the impact of fingolimod, IFNβ-1α, GA, and DMF on *Vsir* expression in the PBMCs of RRMS patients have not been elucidated.

Herein, we studied the expression level of *Vsir* in the PBMCs of patients with RRMS, and then we applied single-cell RNA sequencing to show which cells have altered *Vsir* expression compared to the control. Finally, we investigated the effect of fingolimod, IFNβ-1α, GA, and DMF on the *Visr* expression in the PBMCs of RRMS patients.

2. Materials and methods

2.1. Single-cell transcriptome analysis

The raw data was obtained from the study by Schafflick et al. They performed single-cell RNA sequencing on the blood and cerebrospinal fluid cells of MS patients and controls [11]. The raw data of single-cell RNA sequencing is available in GEO under GSE138266 [13]. The Scanpy is used for data analyses [12]. Cells with the following criteria were included for single-cell RNA sequencing: (I) Each cell must have more than 500 genes and less than 17500 gene expression counts. (II) Less than 20% of counted genes must be related to mitochondrial genes. Normalized expression was computed using the scran package on Bioconductor (http://bioconductor.org/packages/scran). The batch correction was performed to batch effects from 10 samples that were loaded.

To facilitate unsupervised clustering, principal component analysis (PCA) was used to reduce the dimensionality of the top 4000 highly variable genes (HVGs). Clustering was performed on the HVGs data, dimensionality reduced by PCA, and embedded into a k-nearest-neighbor graph using Louvain community detection [13]. In the next step, the cell types were defined based on the expression level of marker genes [14]. Afterward, we derived uniform manifold approximation and projection (UMAP) embeddings presented for visualization from this most relative neighbor graph using a minimum distance of 0.5 and a spread of 1.0 [15].

2.2. Experimental study

2.2.1. Study population

RRMS patients and healthy donors who met the following criteria were included in the current research: I) RRMS diagnoses were based on McDonald criteria [16], II) participants were between 20 and 60 years old, III) RRMS patients were treated with IFNβ-1α, or fingolimod, or DMF, or GA for at least three months, and IV) RRMS patients were not treated with other agents. Patients with the following parameters were excluded: I) being diagnosed with either primary or secondary MS, II) prior three-month of corticosteroid treatment, III) having a history of another inflammatory, chronic degenerative CNS disease, and autoimmune conditions. Regarding the criteria as mentioned above, five naïve RRMS patients, forty treated RRMS patients, and nineteen healthy donors were enrolled for this study. All RRMS patients were in remission at the time of sampling, and the ethnicity of the RRMS patients was the same as the ethnicity of healthy controls.

2.2.2. PBMCs isolation, RNA extraction, and complementary DNA (cDNA) synthesis

Following venous blood collection, PBMC isolation was conducted using Ficoll-Hypaque gradients (Lymphodex, InnoTrain, Germany). Total RNA was extracted from PBMCs using TRIzol reagent (GeneALL Biotechnology, Seoul, Korea). Total RNA was measured using UV-spectroscopy at 260 nm, and 1 μg RNA was used for cDNA synthesis by a specific kit (BioFact, South Korea).

2.2.3. Real-time PCR

RT-PCR was performed using a Step-One Plus (Applied Biosystems, Foster City, CA). The generated cDNA was subjected to SYBR green quantitative RT-PCR analysis (BioFACT™ 2X, South Korea). *Vsir* gene expression was studied in all the samples, and *GAPDH* was used as an internal control. The following conditions were used for real-time PCR: initial denaturation 13 min at 95 °C, denaturation 13 s at 94 °C, primer annealing 30 s at 60 °C, and elongation 20 s at 72 °C. The specific primer sequences for *Vsir* is as follows: (F: 5′- GCGGATGGACAGCAACATT -3′, R: 5′- TTGGAGAGTCAGGGACAGGG-3') and *GAPDH* (F: 5′-AAGGTGAAGGTCGGAGTCAAC-3′, R: 5′-GGGGTCATTGATGGCAACAA-3′).

2.2.4. Statistical analyses

Python 3.7 and GraphPad Prism version 7.05 (GraphPad Software, Inc., San Diego, CA) were used to analyze data. Relative gene expression was assessed using the comparative $2^{-\text{delta CT}}$ method. A *p*-value less than 0.05 was considered statistically significant.

3. Results

3.1. Single-cell transcriptome analysis

3.1.1. Differential cell-type proportion analysis

As we showed in our recent studies, we reanalyzed single-cell transcriptome data to classify MS molecular signatures in PBMC subtypes [17]. Based on marker genes, we identified 21 main clusters related to the PBMCs of MS patients and healthy controls (Fig. 1).

3.1.2. Visualization of Vsir gene expression in the PBMCs of MS patients and controls

First, our results have shown that *Vsir* is remarkably expressed in classical monocytes, intermediate monocytes, non-classical monocytes, plasmacytoid dendritic cells (pDCs), myeloid dendritic cells (mDCs), and granulocytes of PBMCs (Fig. 2 A). Besides, our results have shown a

decreasing trend in the mean expression value of *Vsir* in the PBMCs of MS patients compared with the PBMCs of the controls (Fig. 2B). Afterward, we attempted to demonstrate which cells were responsible for the altered *Vsir* expression. Our results have shown that *Vsir* expression is downregulated in classical monocyte, intermediate monocytes, non-classical monocytes, mDC, pDC, and naive B-cells of PBMCs of RRMS patients compared to the control (Fig. 2 C).

3.2. Experimental study

3.2.1. Ex-vivo study

In the current study, we included five treatment-naïve RRMS patients, ten RRMS patients treated with fingolimod, ten RRMS patients treated with DMF, ten RRMS patients treated with IFNβ-1α, ten RRMS patients treated with GA, and nineteen healthy individuals. The clinical information of the included individuals is demonstrated in Table 1.

3.2.2. Vsir expression in the PBMCs of RRMS patients and healthy controls

Our results have shown that *Vsir* mRNA expression is significantly downregulated in the PBMCs of treatment-naïve RRMS patients compared to healthy controls (p-value < 0.01) (Fig. 3 A). Besides, our results have demonstrated that IFNβ-1α, DMF, and GA has significantly increased the mRNA expression of *Vsir* in the PBMCs of RRMS patients (p-value < 0.001, p-value < 0.001, and p-value < 0.0001) (Fig. 3B). However, treatment with fingolimod has not significantly upregulated the mRNA expression of *Vsir* in the PBMCs of RRMS patients (p-value > 0.05) (Fig. 3B).

4. Discussion

Although much progress has been made in treating MS, its pathogenicity is not fully understood [18]. Advances in single-cell RNA sequencing and immune cell mass cytometry have resulted in remarkable progress in understanding microglia and macrophage phenotypic changes in most diseases, like MS [19]. Despite the autoimmunity base of this multifaceted disease, many questions remain unanswered [11]. For instance, the effect of all immune cell populations in MS development has not been fully understood.

Immune checkpoint molecules can considerably regulate immune responses [20]. Growing evidence has indicated the pivotal role of immune checkpoint molecules in the pathogenesis of various autoimmune diseases and cancers [21,22]. Indeed, inhibitory immune checkpoint molecules can maintain self-tolerance and prevent autoimmunity development [23]. Consistent with this, it has been shown that the expression levels of CTLA-4 and programmed death-ligand 1 (PD-L1), as two inhibitory immune checkpoint molecules are substantially down-regulated in the PBMCs of RRMS patients [17]. As a novel inhibitory immune checkpoint molecule, VISTA can also contribute to the regulation of immune responses [24]. It has been shown that *Vsir* knockout can lead to spontaneous stimulation of $CD8^+$ and $CD4^+$ T cells in aged mice, and its knockout can pave the way for the loss of peripheral tolerance [25]. In line with this, Ceeraz et al. have reported that loss of VISTA can lead to the activation of T cells and exacerbate systemic lupus erythematous in animal models [26]. Besides, Le Mercier et al. have shown that VISTA blockade can increase tumor-specific T cells in the peripheral blood and facilitate the infiltration of T cells into the tumor microenvironment [9]. Han et al. have indicated that loss of VISTA can lead to systemic autoimmune diseases, and administrating VISTA agnostic can attenuate the disease and decrease autoantibodies in animal models [27]. Borggrewe et al. have indicated that VISTA is mainly expressed in microglial cells of human and mice brains, and its expression is substantially downregulated in microglial cells of MS tissues [28]. Our in-silico results have shown a decreasing trend in *Vsir* expression in PBMCs of MS patients compared to healthy controls. Also, our ex-vivo results have indicated that *Vsir* expression level is significantly decreased in PBMCs of treatment-naïve RRMS patients compared

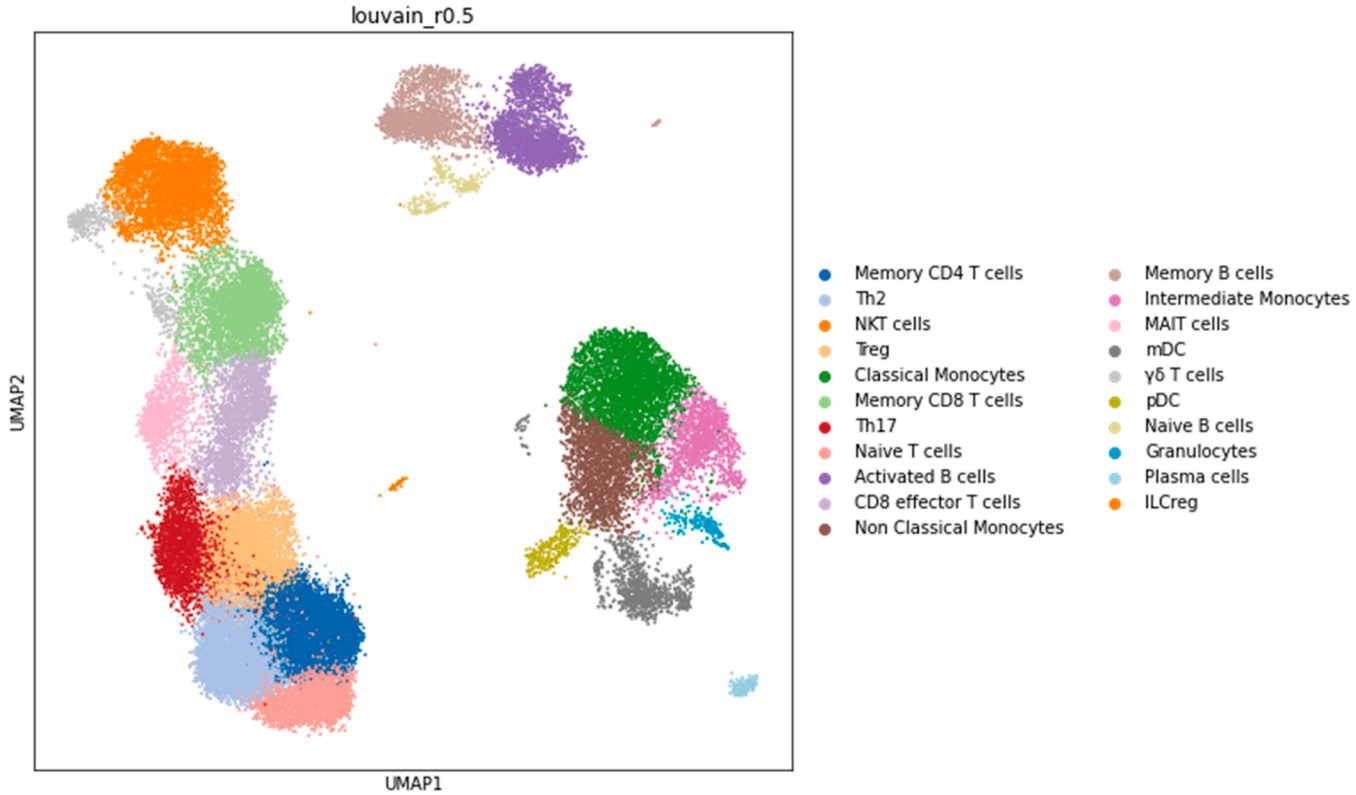

Fig. 1. Louvain clustering and cell annotation were employed to characterize 21 major color-coded cell clusters identified in merged single-cell transcriptomes analysis of PBMCS.

to the healthy controls. Our single-cell RNA sequencing results have demonstrated that the expression of *Vsir* is decreased in classical monocyte, intermediate monocytes, non-classical monocytes, mDC, pDC, and naive B-cells of PBMCs of RRMS patients compared to the control.

The FDA has approved DMF, fingolimod, IFNβ-1α, and GA for patients with MS [29]. GA treatment is associated with the differentiation of T helper type 2 cells and increased frequency of regulatory T cells (Tregs). It has been shown that GA-treated monocytes secrete less pro-inflammatory cytokines and upregulate the expression of anti-inflammatory factors like IL-10 and TGF-β [30]. Besides, it has been reported that GA can inhibit T helper 17 cell-mediated immune responses and decrease IL-1β expression [31]. In line with these, it has been reported that GA treatment can decrease the plasmatic level of IFN-γ and increase the plasmatic level of TGF-β, indicating the anti-inflammatory properties of GA in patients with MS [32]. Our results have shown that RRMS patients treated with GA upregulate *Vsir* expression compared to treatment-naïve RRMS patients.

It has been reported that treatment with IFNβ-1α can substantially decrease mortality over a 21-year period compared to placebo [33]. It has been shown that treatment with IFNβ-1α can upregulate brain-derived neurotrophic factor in vitro [34]. Also, IFNβ-1a-treated myelin-specific T cells can express the high level of immunosuppressive factors, and IFNβ-1a can upregulate IL-10 level in cerebrospinal fluid after two years of treatment [35]. Our results have shown that RRMS patients treated with IFNβ-1α upregulate *Vsir* expression compared to treatment-naïve RRMS patients.

It has been shown that treatment with DMF can considerably decrease the risk of relapse compared to placebo [36]. Hansen et al. have shown that DMF therapy can decrease the frequency of

Table 1
Classification based on age, sex, and medication.

Groups	N (%)	Age (Mean ± SD)	Female N (%)
Healthy people	19	29.89 ± 8.1	11 (57.89%)
Treatment-naïve patients	5	34 ± 5.05	4 (80%)
Fingolimod	10	34.3 ± 6.1	7 (70%)
IFNβ-1α	10	35.1 ± 10.3	7 (70%)
DMF	10	28 ± 6.03	7 (70%)
GA	10	33.7 ± 7.2	7 (70%)

Abbreviations: IFNβ-1α: interferon-beta 1-alpha, DMF: dimethyl fumarate, and GA: glatiramer acetate

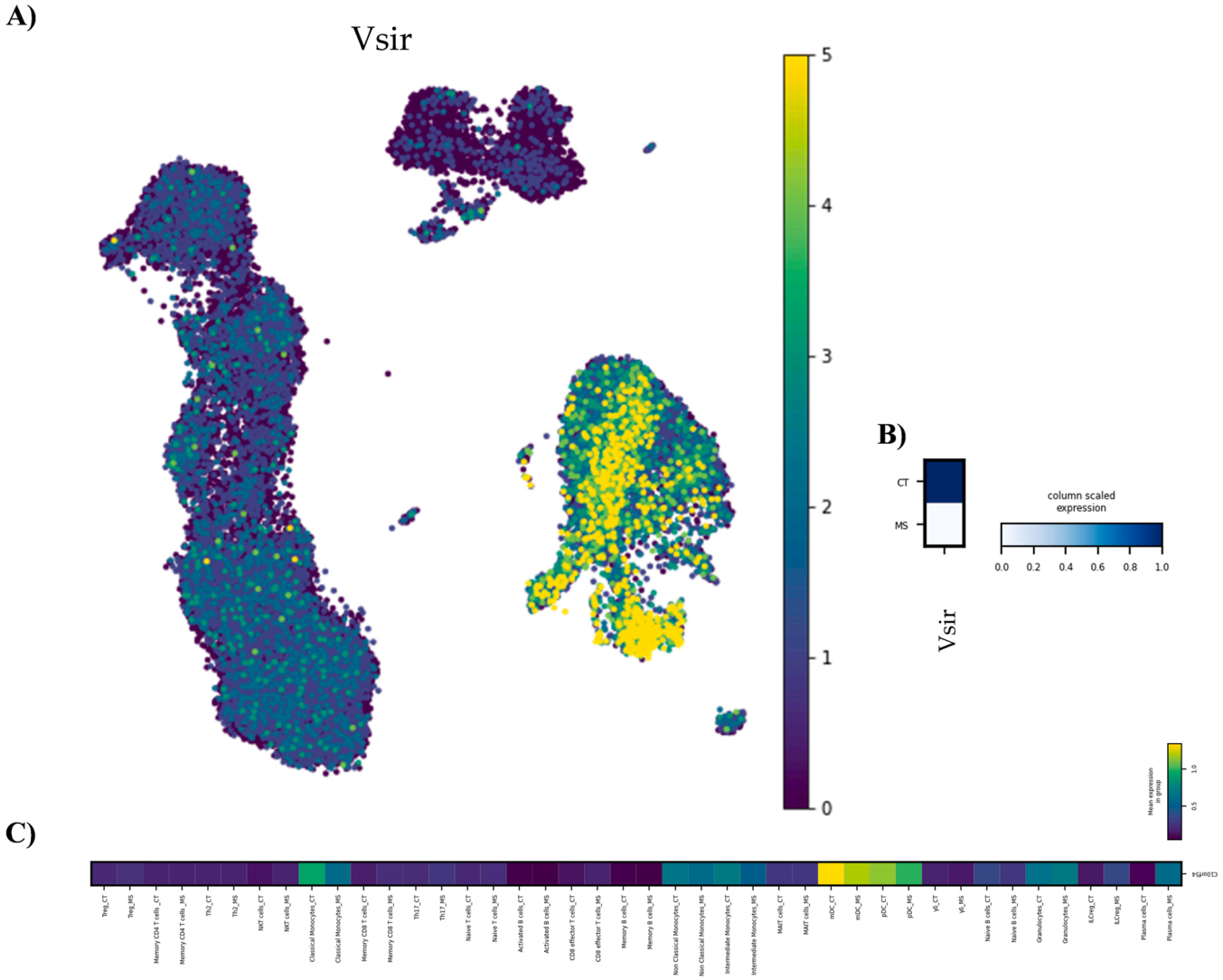

Fig. 2. *Vsir* expressions in the different cell populations. A) Analysis of 21 major cell populations of PBMCs revealed that *Visr* is mainly expressed in classical monocytes, intermediate monocytes, non-classical monocytes, plasmacytoid dendritic cells, myeloid dendritic cells, and granulocytes. B) *Vsir* mean expression in the PBMCs of MS and control. C) *Vsir* expression is downregulated in classical monocyte, intermediate monocytes, non-classical monocytes, mDC, pDC, and naive B-cells of PBMCs of RRMS patients compared to the control. CT: control and MS: multiple Sclerosis.

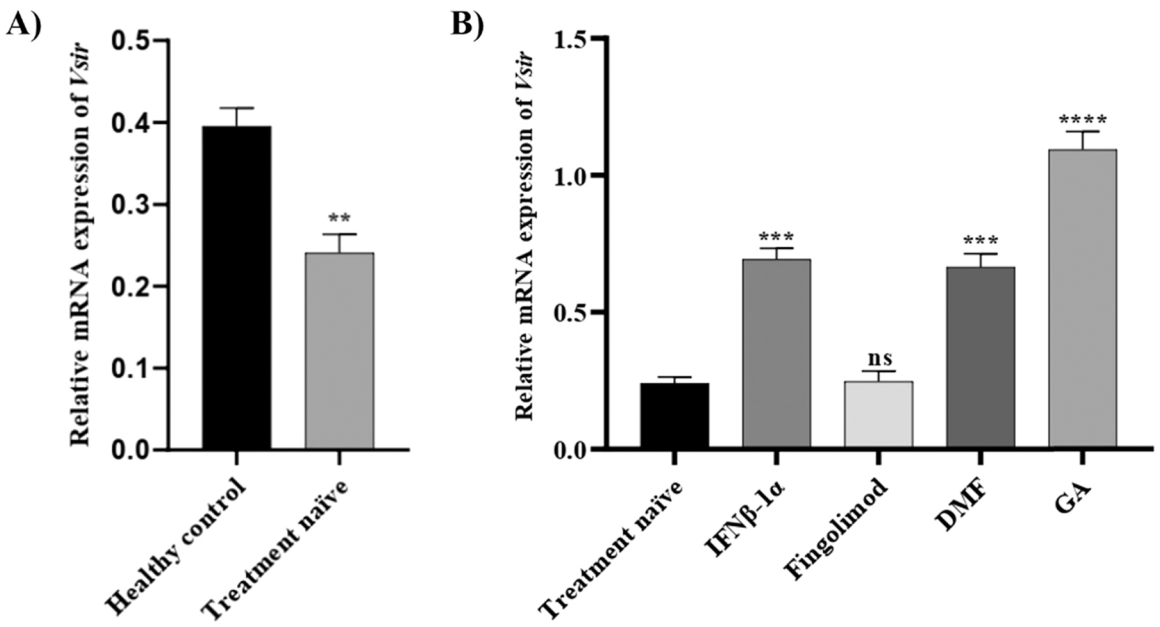

Fig. 3. The expression of *Vsir* mRNA in the PBMCs of healthy controls, treatment-naïve MS patients, and RRMS patients treated with IFNβ-1α, or fingolimod, or DMF, or GA. A) *Vsir* expression is downregulated in the PBMCs of treatment-naïve RRMS patients compared to healthy controls. B) Treatment with IFNβ-1α, or DMF, or GA has upregulated *Vsir* expression in the PBMCs of RRMS patients. *GAPDH* was used as a housekeeping gene; data are expressed as the mean of $2^{-\Delta ct}$ (± SD). * *: *p*-value < 0.01, * ** : *p*-value < 0.001, and * ** *: *p*-value < 0.0001, and ns: non-significant.

pro-inflammatory $CD4^+$ and $CD8^+$ T cells in the circulation. Besides, DMF treatment can reduce T cell proliferation, decrease the differentiation of naïve T cells to effector T cells, and inhibit the migration of $CD4^+$ T cells to CNS [37]. Wu et al. have reported that DMF treatment can decrease the absolute number of $CD4^+$ and $CD8^+$ T cells in patients with RRMS. Also, DMF has substantially decreased specific subsets of T helper 17 and T helper 1 cells and increased a specific subset of anti-inflammatory T helper 2 cells in patients with RRMS [38]. Our results have shown that RRMS patients treated with DMF upregulate *Vsir* expression compared to treatment-naïve RRMS patients.

The current study has several strengths. First, we applied single-cell RNA sequencing analyses to demonstrate *Vsir* mRNA expression in various cell populations of PBMCs of MS patients and controls. Besides, we validated the altered expression of *Vsir* in the PBMCs of RRMS patients and controls. However, our study suffers from several limitations, as well. First, the sample size of treatment-naïve RRMS patients was small. Second, we could not follow the same patients to evaluate the expression level of *Vsir* in the same patients.

5. Conclusion and Suggestion

Our results have indicated that *Vsir* is mainly expressed in classical monocytes, intermediate monocytes, non-classical monocytes, pDCs, mDCs, and granulocytes. Consistent with the observed trend in in-silico results, our experimental results have indicated that *Vsir* expression is substantially downregulated in the PBMCs of treatment-naïve RRMS patients compared to controls. Our single-cell RNA sequencing results have demonstrated that *Vsir* expression is considerably decreased in classical monocyte, intermediate monocytes, non-classical monocytes, mDC, pDC, and naive B-cells of PBMCs of RRMS patients compared to the control. Our results have also shown that *Vsir* expression level has been substantially higher in RRMS treated with IFNβ-1α, or DMF, or GA compared to treatment-naïve RRMS patients. Despite the encouraging results of the current study, further investigations such as isolating and studying the above-mentioned immune cells that have shown decreased expression of *Vsir* can further our knowledge of the function and significance of VISTA in MS pathogenicity. Besides, studying VISTA protein expression level can also provide valuable insights into the significance of this inhibitory immune checkpoint molecule in MS pathogenicity.

Institutional review board statement

The Ethics Committee of Tabriz University of Medical Sciences, Tabriz, Iran, approved this research under the following Ethics code (IR.TBZMED.REC.1399.074).

Informed consent statement

All enrolled individuals were informed and signed the consent form.

Author Contributions

A.D. performed the experiment and analyzed the data. Z.A. and M.A. S wrote the primary version of the manuscript. H.S and A.M.F performed the single sequencing analysis. B.B, M.A.S, and N.J.T, P.L, revised the manuscript. S.R and H.A collected the samples. N.H, H.A, S.P, M.S, and M.G: contributed to English editing of the manuscript and also helped with data categorization. E.S. and V.R: the corresponding authors of the manuscript contributed to the reviewing and supervising the manuscript. All authors have read and agreed to the published version of the manuscript.

Conflict of interest statement

The authors declare no conflict of interest.

References

[1] K.A. McKay, V. Kwan, T. Duggan, H. Tremlett, Risk factors associated with the onset of relapsing-remitting and primary progressive multiple sclerosis: a systematic review, BioMed Res. Int. (2015) (2015).
[2] N. Ghasemi, S. Razavi, E. Nikzad, Multiple sclerosis: pathogenesis, symptoms, diagnoses and cell-based therapy, Cell J. 19 (1) (2017) 1.
[3] D. Ofengeim, N. Giagtzoglou, D. Huh, C. Zou, J. Yuan, Single-cell RNA sequencing: unraveling the brain one cell at a time, Trends Mol. Med. 23 (6) (2017) 563–576.
[4] H. Chen, F. Ye, G. Guo, Revolutionizing immunology with single-cell RNA sequencing, Cell. Mol. Immunol. 16 (3) (2019) 242–249.

[5] M. Kreileder, I. Barrett, C. Bendtsen, D. Brennan, W. Kolch, Signaling dynamics regulating crosstalks between T-cell activation and immune checkpoints, Trends Cell Biol. (2020).
[6] J.L. Lines, L.F. Sempere, T. Broughton, L. Wang, R. Noelle, VISTA is a novel broad-spectrum negative checkpoint regulator for cancer immunotherapy, Cancer Immunol. Res. 2 (6) (2014) 510–517.
[7] J.L. Lines, E. Pantazi, J. Mak, L.F. Sempere, L. Wang, S. O'Connell, S. Ceeraz, A. A. Suriawinata, S. Yan, M.S. Ernstoff, R. Noelle, VISTA is an immune checkpoint molecule for human T cells, Cancer Res. 74 (7) (2014) 1924–1932.
[8] J. Wang, G. Wu, B. Manick, V. Hernandez, M. Renelt, C. Erickson, J. Guan, R. Singh, S. Rollins, A. Solorz, VSIG-3 as a ligand of VISTA inhibits human T-cell function, Immunology 156 (1) (2019) 74–85.
[9] I. Le Mercier, W. Chen, J.L. Lines, M. Day, J. Li, P. Sergent, R.J. Noelle, L. Wang, VISTA regulates the development of protective antitumor immunity, Cancer Res. 74 (7) (2014) 1933–1944.
[10] N. Li, W. Xu, Y. Yuan, N. Ayithan, Y. Imai, X. Wu, H. Miller, M. Olson, Y. Feng, Y. H. Huang, Immune-checkpoint protein VISTA critically regulates the IL-23/IL-17 inflammatory axis, Sci. Rep. 7 (1) (2017) 1–11.
[11] D. Schafflick, C.A. Xu, M. Hartlehnert, M. Cole, A. Schulte-Mecklenbeck, T. Lautwein, J. Wolbert, M. Heming, S.G. Meuth, T. Kuhlmann, C.C. Gross, H. Wiendl, N. Yosef, G. Meyer Zu Horste, Integrated single cell analysis of blood and cerebrospinal fluid leukocytes in multiple sclerosis, Nat. Commun. 11 (1) (2020) 247.
[12] F.A. Wolf, P. Angerer, F.J. Theis, SCANPY: large-scale single-cell gene expression data analysis, Genome Biol. 19 (1) (2018) 1–5.
[13] V.D. Blondel, J.-L. Guillaume, R. Lambiotte, E. Lefebvre, Fast unfolding of communities in large networks, J. Stat. Mech.: Theory Exp. 2008 (10) (2008) P10008.
[14] C. Soneson, M.D. Robinson, Bias, robustness and scalability in single-cell differential expression analysis, Nat. Methods 15 (4) (2018) 255.
[15] E. Becht, L. McInnes, J. Healy, C.-A. Dutertre, I.W. Kwok, L.G. Ng, F. Ginhoux, E.W. J.Nb Newell, Dimensionality reduction for visualizing single-cell data using UMAP, Nat. Biotechnol. 37 (1) (2019) 38.
[16] C.H. Polman, S.C. Reingold, B. Banwell, M. Clanet, J.A. Cohen, M. Filippi, K. Fujihara, E. Havrdova, M. Hutchinson, L. Kappos, F.D. Lublin, X. Montalban, P. O'Connor, M. Sandberg-Wollheim, A.J. Thompson, E. Waubant, B. Weinshenker, J.S. Wolinsky, Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria, Ann. Neurol. 69 (2) (2011) 292–302.
[17] A. Derakhshani, Z. Asadzadeh, H. Safarpour, P. Leone, M.A. Shadbad, A. Heydari, B. Baradaran, V. Racanelli, Regulation of CTLA-4 and PD-L1 expression in relapsing-remitting multiple sclerosis patients after treatment with fingolimod, IFNβ-1α, glatiramer acetate, and dimethyl fumarate drugs, J. Pers. Med. 11 (8) (2021) 721.
[18] J. Correale, M.I. Gaitán, M.C. Ysrraelit, M.P. Fiol, Progressive multiple sclerosis: from pathogenic mechanisms to treatment, Brain 140 (3) (2017) 527–546.
[19] S. Zia, K.S. Rawji, N.J. Michaels, M. Burr, B.J. Kerr, L.M. Healy, J.R. Plemel, Microglia diversity in health and multiple sclerosis, Front. Immunol. 11 (2020) 2895.
[20] N.K. Ahangar, N. Hemmat, M. Khalaj-Kondori, M.A. Shadbad, H. Sabaie, A. Mokhtarzadeh, N. Alizadeh, A. Derakhshani, A. Baghbanzadeh, K.J. Ijoms Dolatkhah, Open accessreviewthe regulatory cross-talk between microRNAs and novel members of the B7 family in human diseases: a scoping review, Int. J. Mol. Sci. 22 (5) (2021) 2652.
[21] K. Dolatkhah, N. Alizadeh, H. Mohajjel-Shoja, M. Abdoli Shadbad, K. Hajiasgharzadeh, L. Aghebati-Maleki, A. Baghbanzadeh, N. Hosseinkhani, N. Karim Ahangar, B. Baradaran, B7 immune checkpoint family members as putative therapeutics in autoimmune disease: an updated overview, Int. J. Rheum. Dis. (2022).
[22] N. Hosseinkhani, A. Derakhshani, O. Kooshkaki, M. Abdoli Shadbad, K. Hajiasgharzadeh, A. Baghbanzadeh, H. Safarpour, A. Mokhtarzadeh, O. Brunetti, S.C. Yue, Immune checkpoints and CAR-T cells: the pioneers in future cancer therapies? Int. J. Mol. Sci. 21 (21) (2020) 8305.
[23] C. Paluch, A.M. Santos, C. Anzilotti, R.J. Cornall, S.J. Davis, Immune checkpoints as therapeutic targets in autoimmunity, Front. Immunol. (2018) 2306.
[24] N. Hosseinkhani, A. Derakhshani, M.A. Shadbad, A. Argentiero, V. Racanelli, T. Kazemi, A. Mokhtarzadeh, O. Brunetti, N. Silvestris, B.J. Baradaran, The role of V-domain Ig suppressor of T cell activation (VISTA) in cancer therapy: lessons learned and the road ahead, Front. Immunol. 12 (2021) 1797.
[25] L. Wang, I. Le Mercier, J. Putra, W. Chen, J. Liu, A.D. Schenk, E.C. Nowak, A. A. Suriawinata, J. Li, R.J. Noelle, Disruption of the immune-checkpoint VISTA gene imparts a proinflammatory phenotype with predisposition to the development of autoimmunity, Proc. Natl. Acad. Sci. 111 (41) (2014) 14846–14851.
[26] S. Ceeraz, P.A. Sergent, S.F. Plummer, A.R. Schned, D. Pechenick, C.M. Burns, R. J. Noelle, VISTA deficiency accelerates the development of fatal murine lupus nephritis, Arthritis Rheumatol. 69 (4) (2017) 814–825.
[27] X. Han, M.D. Vesely, W. Yang, M.F. Sanmamed, T. Badri, J. Alawa, F. López-Giráldez, P. Gaule, S.W. Lee, J.-P. Zhang, PD-1H (VISTA)–mediated suppression of autoimmunity in systemic and cutaneous lupus erythematosus, Sci. Transl. Med. 11 (522) (2019).
[28] M. Borggrewe, C. Grit, W.F. Den Dunnen, S.M. Burm, J.J. Bajramovic, R.J. Noelle, B.J. Eggen, J.D.J.G. Laman, VISTA expression by microglia decreases during inflammation and is differentially regulated in CNS diseases, Glia 66 (12) (2018) 2645–2658.
[29] K. Gohil, Multiple sclerosis: progress, but no cure, Phar. Ther. 40 (9) (2015) 604.
[30] P.H. Lalive, O. Neuhaus, M. Benkhoucha, D. Burger, R. Hohlfeld, S.S. Zamvil, M.S. J.Cd Weber, Glatiramer acetate in the treatment of multiple sclerosis: emerging concepts regarding its mechanism of action, CNS Drugs 25 (5) (2011) 401–414.
[31] M. Melnikov, S. Sharanova, A. Sviridova, V. Rogovskii, N. Murugina, A. Nikolaeva, Y. Dagil, V. Murugin, T. Ospelnikova, A. Boyko, The influence of glatiramer acetate on Th17-immune response in multiple sclerosis, PLoS One 15 (10) (2020), e0240305.
[32] A. Ganji, M.E. Monfared, S. Shapoori, P. Nourbakhsh, A. Ghazavi, K. Ghasami, G. Mosayebi, Effects of interferon and glatiramer acetate on cytokine patterns in multiple sclerosis patients, Cytokine 126 (2020), 154911.
[33] D. Goodin, A. Reder, G. Ebers, G. Cutter, M. Kremenchutzky, J. Oger, D. Langdon, M. Rametta, K. Beckmann, T.J.N. DeSimone, Survival in MS: a randomized cohort study 21 years after the start of the pivotal IFNβ-1b trial, Random. Control. Trial 78 (17) (2012) 1315–1322.
[34] Z. Karmand, H.-P. Hartung, O.J.F.N. Neuhaus, Interferon beta-1a induces expression of brain-derived neurotrophic factor in human T lymphocytes in vitro and not in vivo, Future Med. 15 (1) (2020).
[35] R. Rudick, R. Ransohoff, J. Lee, R. Peppler, M. Yu, P. Mathisen, V.J.N. Tuohy, In vivo effects of interferon beta-1a on immunosuppressive cytokines in multiple sclerosis, Neurology 50 (5) (1998) 1294–1300.
[36] R. Gold, G. Giovannoni, J.T. Phillips, R.J. Fox, A. Zhang, L. Meltzer, N.C.J.M.S. J. Kurukulasuriya, Efficacy and safety of delayed-release dimethyl fumarate in patients newly diagnosed with relapsing–remitting multiple sclerosis (RRMS), Mult. Scler. 21 (1) (2015) 57–66.
[37] R.H. Hansen, H.H. Chow, J.R. Christensen, F. Sellebjerg, M.R.J.M.S. von Essen, Dimethyl fumarate therapy reduces memory T cells and the CNS migration potential in patients with multiple sclerosis, Mult. Scler. Relat. Disord. 37 (2020), 101451.
[38] Q. Wu, Q. Wang, G. Mao, C.A. Dowling, S.K. Lundy, Y.J.T.Jo.I. Mao-Draayer, Dimethyl fumarate selectively reduces memory T cells and shifts the balance between Th1/Th17 and Th2 in multiple sclerosis patients, J. Immunol. 198 (8) (2017) 3069–3080.