Identification of two potential small-molecules that inhibit the CD155/TIGIT pathway in human astrocytoma

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RESEARCH ARTICLE

Identification of Two Potential Small-molecules that Inhibit the CD155/TIGIT Pathway in Human Astrocytoma

Ahmed Qandouci, Khadija E. Azhary, Sanaa Souata, Abdallah Badou

Abstract

Astrocytoma represents a malignant brain tumor. Therapeutic strategies are in continuous development to ameliorate the overall-survival. Several immune checkpoints like CD155 and TIGIT were explored for this purpose. Still, the specific mAb against these molecules showed limited outcome, which supports the need of investigating other strategies to block this pathway such as small-molecules drugs. In this study, bioinformatics approaches were used to answer our question. First, TCGA-database was explored to evaluate the implication of CD155 and TIGIT in the progression of astrocytoma. Then CIBERSORT was used to analyze the difference of immune infiltration depending on TIGIT expression. Finally, a novel drug discovery method, using structure-based docking to find effective small molecules that target CD155/TIGIT pathway was used. Our results report that CD155 and TIGIT are significantly associated with clinical data including age, IDH-status, grade and molecular subtype of glioblastoma. Also, our data indicate that high expression of CD155 and TIGIT are associated to worse overall survival. Furthermore, the CIBERSORT analysis showed the association of this pathway with an inflammatory microenvironment. Finally, the virtual screening allowed the identification of acteoside and rutin, as inhibitor small-molecules to CD155/TIGIT pathway, suggesting their potential to stimulate the immune system against the progression of astrocytoma.

Keywords: Astrocytoma, CD155/TIGIT pathway, Inhibition, Acteoside and rutin

1. Introduction

A strocytomas is a central nervous system (CNS) tumor, that initiates from glial cell. These tumors are considered the most malignant brain tumor, where glioblastoma multiform (GBM) represents the most aggressive subtype with a mean overall survival of 15 months [1]. The development of immunotherapeutic strategies brought lot of hope to patients suffering from tumors including astrocytoma. However, this strategy, based specially on the use on specific monoclonal antibodies didn’t reach expectations of treating this type of glioma even when targeting several molecules. These unsatisfactory results were at the origin of looking after new immunotherapeutic strategies that could have a better impact on the treatment of this tumor.

CD155 is a poliovirus receptor (PVR) that have similar cell adhesion characteristics of the immunoglobulin superfamily [2–4]. Several malignant tumors were shown to overexpress CD155 including lung adenocarcinoma, melanoma, breast cancer and glioma [5–8].

TIGIT (T cell immunoglobulin and ITIM domain) is a transmembrane receptor with an inhibitory
domain expressed on T-cells and NK-cells [9–11]. TIGIT also represents bad prognosis in different tumor sites [12–16], and represents a potential candidate for immunotherapy [17].

Immunotherapy aims to stimulate the immune system against the pathogen. This strategy is usually based on the use of monoclonal antibodies that would block a potential immune checkpoint.

Small-molecules are chemical compounds drugs that have immunomodulatory capacities. They are characterized with their small size that allow them to transfix into the tissues with high efficiency. Previous studies brought evidences that these chemical drugs would be of good benefit to patient in terms of administration and tolerance.

Acteoside (Alpha- l-rhamnosyl-(1→3)-beta-D-glucoside), is a phenylthioanoid glycoside of hydroxytyrosol commonly used in herbal medicines [18]. This molecule plays several pharmacological roles like, anticancer, anti-inflammatory and protective actions [19–21]. Acteoside has shown to have effect on B16 melanoma cells by inhibiting the melanogenesis [22]. Also, the use of Acteoside in murine model of melanoma had a significant impact on the suppression the tumor growth [23].

Rutin (3, 3', 4', 5, 7-pentahydroxyflavone-3-rhamnoglucoside) is molecule that can be used for the modulation of several biological activities including tumor [24,25]. It is a flavonoid compound that is found in multiple plants such as black tea, apples and grapes. In cancer, Rutin was shown to play a critical role as an antitumor molecule [26]. In lung cancer, Rutin was suggested as a potential molecule to increase the expression of TNF-alpha [27]. Also in triple-negative breast cancer, this small-molecule had a significant effect in stopping cell progression by restoring the chemosensitivity of cyclophosphamide [28].

In this study, after showing the significant implication of the CD155/TIGIT pathway in the progression of astrocytoma, we identified an active site on this pathway that could be inhibited by two small-molecules independently: Acteoside and Rutin. Our results suggest that these molecules could be potential alternative to monoclonal antibodies in the inhibition of the CD155/TIGIT pathway.

2. Material and methods TCGA dataset

A total of 346 including 152 Glioblastoma Multiform (GBM) and 194 Low-Grade Glioma (LGG) patients were explored through the cBioportal for Cancer Genomics https://www.cbioportal.org/, by evaluating clinical data and converted Log2 mRNA-seq database of The Cancer Genome Atlas (TCGA).

2.1. Evaluation of immune cell infiltration

The “Cell type Identification By Estimating Relative Subsets Of RNA Transcripts (CIBERSORT)” algorithm (CIBERSORTx (stanford.edu)) was used to investigate the influences of TIGIT expression on immune cell infiltration. Transcriptomic data from advanced grade astrocytoma patients (n = 194) were used to estimate the relative proportions of 22 tumor-infiltrating immune cell types (TIICs) according to the median TIGIT expression level. The LM22 signature matrix with 100 permutations was used for the performance of the deconvolution algorithm. Samples for which the CIBERSORT analysis yielded a p < 0.05 result were used for further analysis. For each sample, the sum of all estimated immune cell type fractions was equal to 1.

2.2. High-throughput virtual screening

2.2.1. Preparation of immune checkpoints for high-virtual screening protein Preparation and Receptor grid generation

The crystal structure of human immunoreceptor T-cell-lg-and-ITIM-domain (TIGIT) in complex with its ligand poliovirus receptor (PVR)/Nectin-like-5/CD155 (PDB ID: 3UDW) was downloaded from protein data bank (http://www.rcsb.org/). To ensure correct starting structures initial structure of the protein was refined and subjected to energy minimization. The protein 3D structure of TIGIT was taken and prepared using the Protein Preparation Wizard in Maestro. Protein was prepared by adding the hydrogen atoms, optimizing hydrogen bonds, removing atomic clashes, Protein was prepared by adding the hydrogen atoms, optimizing hydrogen bonds, removing atomic clashes, adding formal charges to the hetero groups and then optimizing at neutral pH. Finally, the structure was minimized using optimized potential for liquid simulations (OPLS-2005) force field.

2.2.2. Grid generation (binding pocket) in immune checkpoint

Grid generation is essential in molecular docking studies because we must specify the binding pocket in proteins before docking. The active site was determined by literature reported [29] and centroid of the selected residues was used for generation of grid. ‘Receptor Grid Generation’ wizard in Glide was used for grid generation with default parameters for partial cut-off (0.25) and scaling factor (1.0) without any force.

2.2.3. Ligand preparation

For the determination of TIGIT inhibitors in the present examination, 869 small natural molecules with immunomodulatory effect were chosen based
on various literature studies. NCBI's PubChem was used to retrieve all the 3D structures in SDF format of the natural molecules. The molecules were subjected to ligand preparation using Ligand Prep module of Schrodinger suite (Schrodinger). They were optimized with Epik at pH between 7 ± 2, desalting, stereochemistries and tautomer generation were selected to get at least 32 conformations for each ligand and were optimized through OPLS 2005 force field algorithm. The resulting ligands are subjected to high throughput virtual screening.

2.2.4. Virtual screening

The generated grid file with active site residues was used for docking. The ligands were docked by using three phases docking which starts with “High throughput Virtual Screening” (HTVS) followed by “Standard Precision” (SP) and then by “Extra precision” mode (XP). The docked conformers were evaluated using Glide (G) Score. The docked complexes were subjected to Molecular Mechanics/Generalized Born Surface Area (MM-GBSA) analysis for predicting the binding energy by prime approach.

2.3. Statistical analysis

IBM SPSS Statistics (version 26) predictive analytics software was used to check the normality of distribution of each cohort. Statistical analyses and graph designs were generated on GraphPad Prism 8.0 software (GraphPad Software, USA). Mann–Whitney t-test was used to compare differences between medians for the expression of genes. Correlation was performed by Spearman test and ANOVA was used to analyze the differences among means. Log Rank Mantel–Cox test was performed to evaluate the impact of rates of expression of genes on the overall survival. Two independent researchers evaluated the results before validation.

3. Results CD155 and TIGIT have prognostic value with clinical data in human astrocytoma

Before starting our analysis, we wanted to see if our molecules of interest are linked to a bad prognosis in human astrocytoma. We first analyzed the expression of CD155 depending on several clinical data (Table 1) (see Table 2).

<table>
<thead>
<tr>
<th>Clinical parameters</th>
<th>TCGA Cohorts (n)</th>
<th>Median/Mean of CD155mRNA</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women (n = 141)</td>
<td>8.960</td>
<td>0.1386</td>
<td></td>
</tr>
<tr>
<td>Man (n = 205)</td>
<td>9.210</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;40 (n = 113)</td>
<td>8.760</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>&gt;40 (n = 233)</td>
<td>9.506</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>IDH mutation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mutant (n = 40)</td>
<td>8.793</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Wild Type (n = 163)</td>
<td>9.964</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free (n = 179)</td>
<td>9.054</td>
<td>0.0902</td>
<td></td>
</tr>
<tr>
<td>Progressed/Recurred (n = 149)</td>
<td>9.228</td>
<td>0.0902</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Expression of TIGIT depending on clinical data of astrocytoma patients.

<table>
<thead>
<tr>
<th>Clinical parameters</th>
<th>TCGA Cohorts (n)</th>
<th>Median/Mean of TIGIT mRNA</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women (n = 137)</td>
<td>2.322</td>
<td>0.0563</td>
<td></td>
</tr>
<tr>
<td>Man (n = 201)</td>
<td>2.710</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;40 (n = 113)</td>
<td>1.961</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>&gt;40 (n = 233)</td>
<td>2.807</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>IDH mutation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mutant (n = 40)</td>
<td>1.718</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Wild Type (n = 157)</td>
<td>2.897</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free (n = 176)</td>
<td>9.054</td>
<td>0.5420</td>
<td></td>
</tr>
<tr>
<td>Progressed/Recurred (n = 145)</td>
<td>9.228</td>
<td>0.5420</td>
<td></td>
</tr>
</tbody>
</table>

We found that CD155 has no link with the gender ($p = 0.1386$) and is significantly more expressed in higher age >40 compared to lower age <40 ($p < 0.0001$). Also, CD155 has no impact on the disease-free survival status, where the t-test didn't reveal any significant difference between the disease-free status and the disease progressed/recurred status ($p = 0.0902$). Furthermore, CD155 was shown to be associated to the IDH status, where the expression of CD155 was higher in the IDH wild type status compared to the IDH mutated status ($p < 0.0001$). Finally, and concerning the molecular subtype, the expression of CD155 was significantly different between the molecular subtypes of glioblastoma ($p < 0.0001$). These results indicate that CD155 have prognostic value in human astrocytoma.

Concerning the other molecule, results showed that TIGIT also have prognostic value in human astrocytoma, and interestingly in the same parameters where CD155 were reported to be implicated. First, no differences of TIGIT expression were shown concerning

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1 Please check the number of cases by sex and age, which, as in the previous table, should be identical unless data are not available (in which case they should also appear in the table as NA with their number).
the gender ($p = 0.0563$) or the disease-free survival status ($p = 0.5420$). Regarding age, elevated rates of expression of TIGIT were reported in higher age (>40) compared to lower age (<40) with a significant difference ($p < 0.0001$). Also, astrocytoma patients with the IDH wildtype status expressed more TIGIT compared to patient with IDH mutated status ($p < 0.0001$). Additionally, TIGIT expression also was significantly different apropos of the molecular subtype ($p = 0.0023$) indicating its probable implication the progression of human astrocytoma.

3.1. CD155 and TIGIT express their highest levels in the advanced grade of astrocytoma and are positively correlated

Since CD155 and TIGIT have prognostic value with clinical data of astrocytoma patients, we wanted to see if they are also involved in the progression of this tumor. First, in (Fig. 1a), CD155 had its higher rates of expression in astrocytoma grade-IV compared to astrocytoma grade-II and astrocytoma grade-III ($p < 0.0001; p < 0.0001$ respectively), but no
significant difference was reported between the groups of grade-II and grade-III ($p = 0.1251$). Also, in (Fig. 1b), grade IV of astrocytoma expressed elevated rates of TIGIT compared to grade-II and grade III ($p < 0.0001$; $p < 0.0001$ respectively). Here again levels of expression of TIGIT weren't significantly different between the groups of grade-II and grade-III ($p = 0.0838$). Add to this, Spearman r test showed that CD155 and TIGIT are positively and significantly correlated in human astrocytoma ($r = 0.1705$; $p = 0.0016$) (Fig. 1c). These results suggest that this pathway represents bad prognosis, and is incriminated in the progression of astrocytoma.

### 3.2. High expression of CD155 and TIGIT represents bad prognosis regarding the overall survival

In terms of survival, Log Rank Mentel–Cox test was performed to evaluate the impact of expression of CD155 and TIGIT on the survival for astrocytoma patients (Fig. 2). In (Fig. 2-a), patients were stratified to two cohorts depending on the median of expression of CD155. The test revealed that patients having low rates of CD155 had a better overall survival compared to patients having high rates of expression of CD155 ($p < 0.0001$). Concerning the impact of expression of TIGIT on the overall survival (Fig. 2-b), the result reported that astrocytoma patients expressing higher levels of TIGIT had poor overall survival prognostic compared to patients expressing lower levels of TIGIT. Add to this, the upregulation of both CD155 and TIGIT in the same patient had worse prognostic in terms of the overall survival compared to patients expressing lower rates of both CD155 and TIGIT (Fig. 2-c). This supports our hypothesis that this pathway represents a state of malignancy in astrocytoma patients when both molecules are highly expressed.

![Fig. 2](image-url) Survival analysis depending on the expression of TIGIT and CD155 in human astrocytoma. a) Survival analysis depending on the expression of high and low CD155 ($n = 173 * 2$) b) Survival analysis depending on the expression of high and low TIGIT ($n = 170 * 2$) c) Survival analysis depending on the expression of both CD155 and TIGIT ($LL (n = 101)$; $LH (n = 72)$; $HL (n = 72)$; $HH (n = 101)$) The **** stands for a significant statistical analysis which corresponds to a $p < 0.0001$. L for low & H for High.
3.3. High expression of TIGIT is associated to the immune infiltration in human astrocytoma

Since TIGIT was linked to an aggressive malignant astrocytoma, we further wanted to figure out whether the expression of TIGIT was related to tumor infiltrating immune cells (TIICs) depending on the versatile computational method CIBERSORT. As shown in Fig. 4 and 22 subpopulations of immune cells were analyzed depending on high and low expression of TIGIT. The validation of the method has been confirmed on gene expression profiles measured by RNA-sequencing. Transcriptomic data from advanced grade astrocytoma patients (∙ = 194) were divided into 2 groups according to the median, in high and low TIGIT. And in order to allow sensitive and specific discrimination of the 22 immune cell phenotypes, the LM22 gene signature with 100 permutations was used. Furthermore, for each sample, the sum of all estimated immune cell type fractions was equal to 1. CIBERSORT provides a score that can be compared between samples and cell types with a p-value for the deconvolution of each sample, and only samples with a p < 0.05 were used for the following analyses. The fractions of 22 subtypes of TIICs are clearly presented in (Fig. 3).

Statistical tests indicate a significant association between TIGIT expression and the level of infiltration of regulatory T cells, resting NK cells and resting dendritic cells. The three immune subtypes shared a higher percentage in the context of high TIGIT expression compared with to the low TIGIT expression group. Indicating that TIGIT is associated to an inflammatory microenvironment.

4. Virtual screening and identification of small molecule inhibitors

To identify natural molecule inhibitors, we have performed Virtual screening using prepared 869 small natural immunomodulatory molecules against active site residues of TIGIT (where PVR binds on TIGIT). The docking-based screening was performed multi-tiered screening protocol, starting with HTVS followed by SP and XP methods. A total of 35 compounds obtained from XP method were shortlisted based on docking score less than –6.0. A low glide score indicated a high binding affinity towards the target. Then we studied the interactions of the 35 pre-selected compounds with the three motifs conserved on TIGIT of the TIGIT/PVR complex (residues 54–56; 66–74; 112–114). Five compounds were selected based on docking, MM-GBSA scores, and interaction residues (Table 3). By taking into account the main interaction points critical for the formation of the TIGIT/PVR complexes (Q56, N70, G74 and Y113) we suggest two potential inhibitors of TIGIT/PVR complexes, Acteoside and Rutin.

The docking score energy which generated by the binding of Acteoside to the TIGIT, was obtained as –8.191 kcal/mol, that of Rutin was obtained at –6.668 kcal/mol (Table-3). The Acteoside, which binds with the amino acids located in the active region of TIGIT, was attached to the protein by hydrogen bonds; GLN56, GLY74, HIS111 and THR112 residues (Fig. 4). On the other hand, Rutin was attached to the protein by hydrogen bonds; GLN53, THR55, ASN70, ASP72 and TYR113 residues (Fig. 5). The ΔG bind between TIGIT and each
ligand, respectively, were calculated using the MMGBSA and are shown in Table 3. Rutin showed the maximum $\Delta G_{\text{bind}}$ ($-49.92$) and the Acteoside showed the minimum $\Delta G_{\text{bind}}$ ($-38.08$).

5. Discussion²,³

Astrocytoma are the most aggressive brain tumor, and the grade IV (glioblastoma multiform), represents very bad prognosis with an overall survival of only 15 months [1,30]. In this work we aimed to identify a potential molecule that would interrupt the interaction between $\text{CD155}$ and $\text{TIGIT}$, a pathway implicated in the immune suppression, in the purpose of stimulating the immune system against the tumor.

We started by evaluating the levels of expression of $\text{CD155}$ and $\text{TIGIT}$ with several clinical parameters in astrocytoma patients, and found that, beside the significant association of high expression of $\text{CD155}$ and high expression of $\text{TIGIT}$ with age, IDH status and the molecular subtype, $\text{CD155}$ and $\text{TIGIT}$ were also associated to grade, where grade IV astrocytoma patients represented the highest rates of expression of $\text{CD155}$ compared to grade II and grade III, and highest rates of $\text{TIGIT}$ compared to grade II and grade III. Additionally, High expression of $\text{CD155}$ and high expression of $\text{TIGIT}$ were both associated to worse overall survival. Furthermore, patients who had elevated rate of $\text{CD155}$ and $\text{TIGIT}$ were condemned to less overall survival months compared to those expressing lower levels of both molecules. These results indicate that $\text{CD155}$ and $\text{TIGIT}$ are linked to malignant progression of tumor.

Interestingly, when we evaluated the immune infiltration regarding the expression of $\text{TIGIT}$, we found that high expression of $\text{TIGIT}$ was significantly associated to higher rates of regulatory T-cells in the astrocytoma microenvironment, supporting its immunosuppressive role [31–33]. The presence of high rates of resting NK cells in high TIGT conditions would support that they are likely inhibited by the presence of immune checkpoints at their surfaces [34]. Also, these molecules can be activated to exercise their lytic activities following a stimulation with dendritic cell via the NKp30

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² it is not necessary to put the p-value results in the discussion section.
³ it would be interesting to add your considerations on the strengths and limits of your work in this section.
receptor [35]. Finally elevated levels of resting dendritic cells could be the reason of the tolerance to peripheral CD8 T cells as what it was shown with PD-1 and CTLA-4 [36].

Our work is not the first one to show that the CD155/TIGIT pathway is implicated in tumor malignancy. Other study showed that this pathway represents bad prognosis in several tumor sites including melanoma, digestive cancers, hepatocellular carcinoma, cervical cancer and head and neck squamous cell carcinoma [37–42].

Overexpression of TIGIT is reported in various human diseases including cancer, and the inhibition of this immune cell checkpoint might be used to treat various human diseases. In this study, we have screened 869 natural compounds against TIGIT by high throughput virtual screening, based on docking and binding energy scores. It was previously described that the CD155-TIGIT receptor-ligand interacts via three conserved motifs: (V/I) (S/T) Q (residues 54–56 on TIGIT, 61–63 on CD155), AX6G (residues 66–74 in TIGIT, 76–83 in CD155), and T(F/Y)P (residues 112–114 in TIGIT and residues 127–129 in CD155). It was also reported that TIGIT point mutants Q56A and Q56R in the (V/I) (S/T)Q motif, N70R, N70A, G74A in AX6G, and Y113R and Y113A in the T(F/Y)P region weaken or abrogate binding to CD155. Taken together, the
lock-and-key trans-interactions between TIGIT and CD155 are the main interaction points and critically require the “key” motif T(F/Y)P on both CD155 and TIGIT for trans-complex formation. By taking into account all of its results we suggest two potential inhibitors of TIGIT/CD155 complexes, Acteoside and Rutin. Acteoside is a phenylethanoid glycoside widely distributed in many medicinal plants. Studies have shown that Acteoside can exert various biological activities, including its antitumor activity in prostatic cancer, colorectal cancer, melanoma, hepatocellular carcinoma. Similarly, and in addition to its wide beneficial effects, Rutin has been shown to play an anticancer and immunomodulatory role in various types of cancer. Rutin exerts its anti-cancer effect by inducing G2/M arrest and apoptosis in breast cancer MCF-7 cell line. It has also shown anti-tumor properties in colon cancer, lung cancer and prostate cancer. This would suggest that the proposed therapeutic strategy could be valid for several tumor sites.

The methodology Protein–Ligand Docking uses the crystallized structure of a protein to predict how the compounds would interact with the binding site. The protein structures may exhibit some problems such as missing residues or hydrogen atoms, incomplete side chains etc. The algorithm predicts compounds able to interact with the binding site. This does not imply that the actual binding mode of the compound is part of the prediction. This main objective of this approach is
generating hypotheses and not to be used as a definitive proof. Therefore, results obtained from virtual screening should be confirmed in vitro and in vivo in future studies.

6. Conclusion

To conclude, the CD155/TIGIT pathway seems to contribute to immune evasion and make them a potential therapeutic target in human astrocytoma. Our research has provided two potential small molecule inhibitors of the CD155/TIGIT pathway. The use of acteoside and Rutin would be an interesting alternative strategy to monoclonal antibodies to block this pathway for astrocytoma patients.

Author contributions

All the authors cooperated sufficiently to take part of the content. A.Q. designed the study, collected, analyzed and interpreted data, also wrote the manuscript. K.E-A. collected, analyzed data and wrote the manuscript, S. S. collected, analyzed data and wrote the manuscript, A. B. supervised the study, analyzed and interpreted data and revised the final draft of the manuscript. All authors approved the final version of the manuscript.

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Informed consent statement

Not applicable.

Data availability statement

Datasets analyzed during the current study are freely available via https://www.cbioportal.org/.

Conflict of interest

The authors declare no conflict of interest in this work.

Acknowledgment

Not applicable.

Abbreviation

ANOVA Analysis Of Variances
CD Cluster of Differentiation
CTLA-4 Cytotoxic T-Lymphocyte-Associated protein 4
GBM Glioblastoma Multiform
G-CIMP Glioma CpG Island Methylator Phenotype
IDH Isocitrate Dehydrogenase LGG: Low Grade Glioma
mRNA messenger Ribonucleic Acid
NK Natural Killer
SPSS Statistical Package for the Social Science
TCGA The Cancer Genome Atlas
TIGIT T-cell immunoglobulin and ITIM domain

Appendix A. Dataset

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For the determination of TIGIT inhibitors in the present examination, 869 small natural molecules with immunomodulatory effect were chosen based on various literature studies. NCBI’s PubChem was used to retrieve all the 3D structures in SDF format of the natural molecules. The molecules were subjected to ligand preparation using Ligand Prep module of Schrodinger suite (Schrodinger). They were optimized with Epik at pH between 7 and 7.4, subjected to ligand preparation using Ligand Prep (Schrodinger). They were selected to get at least 32 conformations for each ligand and were optimized through OPLS 2005 force field algorithm. The resulting ligands are subjected to high throughput virtual screening.

d. Virtual screening

The generated grid file with active site residues was used for docking. The ligands were docked by using three phases docking which starts with “High throughput Virtual Screening” (HTVS) followed by “Standard Precision” (SP) and then by “Extra precision” mode (XP). The docked conformers were evaluated using Glide (G) Score. The docked complexes were subjected to Molecular Mechanics/ Generalized Born Surface Area (MM-GBSA) analysis for predicting the binding energy by prime approach.

References


