Analysis of the cancer genome atlas (TCGA) database identifies an inverse relationship between interleukin-13 receptor α1 and α2 gene expression and poor prognosis and drug resistance in subjects with glioblastoma multiforme

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Abstract
Glioblastoma multiforme (GBM) is the most common primary brain tumor in adults. A variety of targeted agents are being tested in the clinic including cancer vaccines, immunotoxins, antibodies and T cell immunotherapy for GBM. We have previously reported that IL-13 receptor subunits α1 and α2 of IL-13R complex are overexpressed in GBM. We are investigating the significance of IL-13Ra1 and α2 expression in GBM tumors. In order to elucidate a possible relationship between IL-13Ra1 and α2 expression with severity and prognoses of subjects with GBM, we analyzed gene expression (by microarray) and clinical data available at the public The Cancer Genome Atlas (TCGA) database (Currently known as Global Data Commons). More than 40% of GBM samples were highly positive for IL-13Ra2 mRNA (Log2 ≥ 2) while only less than 16% samples were highly positive for IL-13Ra1 mRNA. Subjects with high IL-13Ra1 and α2 mRNA expressing tumors were associated with a significantly lower survival rate irrespective of their treatment compared to subjects with IL-13Ra1 and α2 mRNA negative tumors. We further observed that IL-13Ra2 gene expression is associated with GBM resistance to temozolomide (TMZ) chemotherapy. The expression of IL-13Ra2 gene did not seem to correlate with the expression of genes for other chains involved in the formation of IL-13R complex (IL-13Ra1 or IL-4Ra) in GBM. However, a positive correlation was observed between IL-4Ra and IL-13Ra1 gene expression. The microarray data of IL-13Ra2 gene expression was verified by RNA-Seq data. In depth analysis of TCGA data revealed that immunosuppressive genes (such as FMOD, CCL2, OSM, etc.) were highly expressed in IL-13Ra2 positive tumors, but not in IL-13Ra2 negative tumors. These results indicate a direct correlation between high level of IL-13R mRNA expression and poor patient prognosis and that immunosuppressive genes associated with IL-13Ra2 may play a role in tumor progression. These findings have important implications in understanding the role of IL-13R in the pathogenesis of GBM and potentially other cancers.

Keywords GBM (glioblastoma multiforme) · TCGA (the cancer genome atlas) · IL-13Ra1 · IL-13Ra2 · Biomarker

Introduction
Glioblastoma multiforme (GBM) is a devastating brain tumor with extremely poor prognosis because of its diffusive and infiltrative nature, which is marked cytological heterogeneity. It is one of the most aggressive and common malignant brain tumors accounting for more than 50% of all gliomas [1]. GBM tumors are histologically and molecularly diverse, exhibiting heterogeneity both between patients and within individual tumors [2]. Several distinct GBM subtypes have been identified based on the gene expression-based molecular classification. Three glioblastoma subtypes were defined based on patient prognosis and gene expression
clustering; proneural, proliferative, and mesenchymal subtypes [3]. Subsequently, gene expression studies from The Cancer Genome Atlas (TCGA) dataset defined four distinct glioblastoma subclasses, including proneural, neural, classical, and mesenchymal [4]. Kim et al. classified GBMs into three prognostic groups, which were different from the previously identified subtypes, and identified a 42 probe set of gene signatures, which associated with tumor aggressiveness and related with the epithelial-mesenchymal transition (EMT) process [5].

Currently, the standard treatment for GBM includes surgical resection followed by radiation therapy, chemotherapy, or a combination of these therapies. Despite the use of these therapies, the median survival time in patients remains dismal, at approximately 1 year. GBM remains an incurable disease with few therapeutic advances over the past several decades. Novel approaches are under development targeting glioma cell surface antigens and receptors, tumor invasion, angiogenesis, proliferation, immune escape, and tumor recurrence [6]. A relatively new form of therapy involves tumor targeting, which relies on the identification of unique or over-expressed cell surface receptors or antigens on tumor cells. One of the extensively studied cell-surface targets is interleukin-13 receptor alpha-2 chain (IL-13Ra2). This receptor is one of the two chains of the IL-13R complex [7]. IL-13Ra2 binds IL-13 with high affinity and has received significant attention in brain tumor therapy because it is overexpressed by high-grade glioblastomas, but not expressed at significant levels by normal brain tissue [6–8]. In addition, it has been shown that IL-13Ra2 promotes tumor invasion and metastasis in mouse models of human pancreatic and ovarian cancers [9, 10]. It is also shown that IL-13Ra2 can protect tumor cells from apoptosis thereby contributing to tumor growth [11]. The IL-13Ra2 chain has become a new and attractive target in immunotherapy using monoclonal antibodies [12], IL-13Ra2-peptide pulsed dendritic cells [13], and IL-13R-targeted chimeric antigen receptor modified T cells [14–16]. To target IL-13R, we have developed a recombinant fusion protein composed IL-13 and a mutated form of Pseudomonas exotoxin (IL-13-PE) [17]. The IL-13-PE was found to be highly selective and potent in killing human GBM cells in vitro and in animal models of glioma tumors [18–22]. Based on these preclinical studies, several Phase I/II clinical trials targeting IL-13Ra2 in GBM by IL-13-PE were undertaken (https://clinicaltrials.gov). A randomized controlled Phase 3 clinical trial was completed [23] and additional clinical trials are planned.

Despite advances in the understanding of IL-13Ra2 biology in glioma tumors and clinical trials targeting this receptor for therapy, the functional significance of IL-13Ra2 expression in malignant glioblastoma is not well understood. We and others have shown that IL-13Ra2 may be associated with the increase in glioma malignancy grade and associated with poor patient prognosis [8, 24, 25]. In order to demonstrate a possible correlation between IL-13Ra1 and a2 expression in GBM with the clinical outcomes, we analyzed datasets publicly available at NCI’s TCGA database, which was established by NCI/NIH to generate the comprehensive catalog of genomic abnormalities (https://tcga-data.nci.nih.gov/tcga/). The TCGA data provided detailed clinical information of a large number of GBM patients. These datasets were downloaded and an association between expression of IL-13Ra2 and clinical outcomes in GBM patients was studied. We also examined a possible association between expression of IL-13Ra1 and clinical outcomes in GBM patients. Our analysis found that the level of IL-13Ra1 and a2 expression is associated with poor patient survival, particularly long-term survival and GBM recurrence. Furthermore, some immune regulatory genes seem to be associated with IL-13Ra2 expression. Our findings have important implications in the understanding of the role of IL-13R in pathogenesis and evaluating possible therapeutic interventions for patients suffering from GBM.

**Materials and methods**

**TCGA data description**

The publicly available TCGA datasets were directly downloaded from the TCGA Data Portal at https://tcga-data.nci.nih.gov/tcga/. The detailed information of the TCGA data structures can be reviewed at https://tcga-data.nci.nih.gov/tcga/tcgaDataType.jsp. The detailed information of the microarray and RNA-Seq experiments, protocols, and software used can be found at the TCGA Data Portal at https://tcga-data.nci.nih.gov/tcga/. For gene expression data, we selected the level-3 microarray dataset, in which Agilent 244K (G4502A), a custom designed microarray platform, was used in the experiments. The microarray data was normalized by Lowess method and presented as calculated Log2 ratio. Additional information regarding the level of the data and methods used in the process can be found at TCGA website (https://tcga-data.nci.nih.gov/tcga/). For RNA-Seq data sets, we selected the level 3 RNA-Seq data which was produced on Illumina HiSeq 2000 sequencers. The RNA-Seq gene expression level 3 data contain Counts which are simply the number of reads overlapping a given gene. The total number of reads for a given transcript is proportional to the expression level of the transcript. Both microarray and RNA-Seq datasets were generated by the laboratories located at University of North Carolina at Chapel Hill. A total of 595 GBM and 10 normal brain microarray data files, 163 RNA-Seq data files, and corresponding clinical data files were downloaded from TCGA website on Sept 27, 2013. These microarray datasets for GBM samples have...
Data transformation and classification

The Agilent gene expression microarray data downloaded from TCGA is presented as log2 ratio of GBM/HuRNA, or Normal brain/HuRNA. In the microarray experiments, Agilent HuRNA (Human universal reference RNA; previously Stratagene HuRNA) was used as a common reference to calculate Log2 ratio. The HuRNA is composed of total RNA pooled from 10 human cancer cell lines. In order to eliminate the potential bias by using the HuRNA as the common reference, we first transformed the original Log2 ratio of GBM/HuRNA to Log2 ratio GBM/Normal brain by using the following formula:

\[
\text{Log2 ratio(GBM/normal brain)} = \text{Log2 ratio(GBM/HuRNA)} - \text{Log2 ratio(normal brain/HuRNA)}
\]

Since:

\[
\text{Log2 ratio(GBM/HuRNA)} = \text{Log2 ratio(normal brain/HuRNA)} - \text{Log2 ratio}[\text{GBM/HuRNA}/\text{(normal brain/HuRNA)}] = \text{Log2 ratio (GBM/normal brain)}
\]

Here, the Log2 ratio (normal brain/HuRNA) is the mean value of log2 ratios from the 10 normal brain data files. Then, we used the transformed gene expression data, which was the Log2 ratio of GBM compared to Normal brain, in the rest of our study [26].

In the TCGA datasets, each clinical dataset represented a unique patient case. Survival was defined as the time interval from the date of surgery to the date of death. In order to elucidate a possible correlation between IL-13Rα2 gene expression and the clinical outcome, we only selected patients with survival > 30 days, indicating that the patient survived from the initial surgery and radiation treatments. A total of 428 GBM gene expression data files having clinical data satisfied the condition for the further gene expression and survival analysis.

By utilizing the transformed datasets, we classified the TCGA GBM tumors into three groups based on the level of IL-13Rα2 gene expression. Of 428 GBM tumors studied, 128 cases (29.9%) were classified into group I, which did not express IL-13Rα2; 328 cases (76.6%) were identified in group II, which expressed IL-13Rα2 with Log2 ratio of > 0 and < 2; and 67 cases (15.7%) were in group III, which was defined as the IL-13Rα2 highly expressed group with Log2 ratio of IL-13Rα2 ≥ 2 (Fig. 1a).

Statistics analyses

An independent t test was performed to calculate the difference between groups. Kaplan–Meier survival analysis was performed to compare the survival distribution between different groups by using GraphPad Prism software (Version 5, GraphPad software Inc., San Diego, CA). A plot of the Kaplan–Meier analysis with appropriate sample size provides the information on the length of survival, median survival time of the distinct sample populations, and significance of the difference between the survival curves.

Results

Patient characteristics

We used the publically available GBM dataset in TCGA database as our primary source of clinical information. Each enrolled subject in this dataset had undergone a surgical resection of GBM and received radiation and some form of chemotherapy. Gene expression data was generated from surgical tumor samples. Age distribution of 428 GBM
subjects was classified and shown in Supplementary Fig S1. Highest number of patients (56%) is in age ranging between ≥50 and <70-years (Supplementary Fig. S1a). There was a clear gender difference in this age group; the number of male patients with GBM was double compared to number of female patients. However, there was no significant difference between the number of male and female patients in any other age groups (Supplementary Fig. S1b). Patient survival data indicated that 79.2% of patients died within 2 years of diagnosis, including 46.5% of patients died within 1 year. Only about 20% of GBM subjects survived more than 2 years (Supplementary Fig. S2).

**High IL-13Rα2 expression is associated with poor prognosis**

To investigate whether *IL-13Ra2* expression is associated with patient prognosis, Kaplan–Meier survival analysis was performed to assess survival of patients with high *IL-13Ra2* and low or no *IL-13Ra2* expressing GBM tumors. A few samples in each group, which have vital status labeled as “Alive”, were eliminated from the dataset for survival analysis. Subjects with GBM that highly expressed *IL-13Ra2* mRNA (Log2 ratio ≥ 2) had a median survival of 335 days (n = 175) compared to 384 days for subjects with *IL-13Ra2* negative tumors (n = 122) (Fig. 2a). The median survival time between *IL-13Ra2* high and *IL-13Ra2* negative groups was statistically significant (p < 0.008). However, there was no significant difference in median survival between patients with *IL-13Ra2* low (n = 113) and *IL-13Ra2* negative tumor groups (Supplementary Fig. S3a). Subjects with *IL-13Ra2* low expression had a significantly higher median survival time of 404 days compared to 335 days for subjects with *IL-13Ra2* high tumors (Supplementary Fig. S3b). Furthermore, when subjects with *IL-13Ra2* low (n = 113) and *IL-13Ra2* negative (n = 122) tumor groups were combined together (n = 235), the median survival was significantly higher compared to subjects with high *IL-13Ra2* expressing tumors (p < 0.003) (Fig. 2b). These results indicate that subjects with higher level of *IL-13Ra2* expression will have poorer survival demonstrating an inverse relationship between *IL-13Ra2* expression and overall survival.

Although the criteria used to define recurrent GBM remain ambiguous due to the varied presentation of new lesions, we selected the recurrent cases from the “days to tumor recurrence” section listed in the TCGA GBM clinical dataset. We next investigated whether *IL-13Ra2* expression is associated with GBM recurrence. Subjects with highly expressed *IL-13Ra2* (Group III, Log2 ratio ≥ 2, n = 33) had a median days to recurrence of 196 compared to 268 days for subjects with *IL-13Ra2* negative tumors (n = 30) (Supplementary Fig. S4a), although the difference between these two groups was not statistically significant (p = 0.065). It is thus possible that subjects with *IL-13Ra2* negative expression will have delayed tumor recurrence compared to subjects with *IL-13Ra2* high expressing tumors and needs to be confirmed in larger sample database.

We also assessed whether *IL-13Ra2* expression was associated with a long-term patient survival. A long-term survival was defined as subject who survived for at least 3 years after diagnosis. A total of 48 subjects survived more than 3 years. Among these, subjects with highly expressed *IL-13Ra2* had a median survival of 1347 days (n = 16) compared to 1796 days for subjects with *IL-13Ra2* negative tumors (n = 14) (Supplementary Fig. S4b). Although the limited numbers of samples prevent achieving statistical significance (p = 0.1241), our findings suggest a tendency that patients with *IL-13Ra2* negative tumors may live longer.

When *IL-13Ra2* expression was compared between lower-grade glioma and high-grade GBM, both *IL-13Ra1*
and IL-13Ra2 gene expression levels were significantly lower in lower-grade glioma compared with GBM. These results suggest that IL-13Ra2 gene expression may also be associated with GBM malignancy grade (Supplementary Fig S5).

High IL-13Ra2 expression in GBM is associated with potential temozolomide resistance

Temozolomide (TMZ) is a most effective chemotherapeutic agent for GBM. Since IL-13Ra2 is highly expressed in GBM but not in normal brain, we investigated the potential association between IL-13Ra2 expression and temozolomide response in GBM patients. We performed Kaplan–Meier analysis to assess the overall survival of subjects with highly expressed IL-13Ra2 or no expression when treated with temozolomide. Subjects with high IL-13Ra2 expressing tumors when treated with temozolomide had a median survival of 435 days (n = 108) compared to 475 days (n = 68) in subjects with IL-13Ra2 negative tumors treated with temozolomide (Fig. 3a). Although the difference between two groups was small, it was statistically significant (p < 0.05). However, in subjects with survival time more than 1 year, this difference became clearer. The subjects with highly expressed IL-13Ra2 (Log2 ratio ≥ 2) had a median survival of 526 days (n = 66) compared to 790 days (n = 33) for subjects with IL-13Ra2 negative tumors. The difference of the median survival between IL-13Ra2 high and IL-13Ra2 negative expression groups was highly statistically significant (p < 0.003) (Fig. 3b). In addition, the similar median survival results were obtained between the patients group with IL-13Ra2 low tumor and the patients group with IL-13Ra2 high tumor treated with temozolomide (Supplementary Fig. S6a-d). Thus subjects with negative IL-13Ra2 tumors had better temozolomide responses and significantly longer survival than those subjects with high IL-13Ra2 expressing tumors. We also performed a detailed data analysis to determine the survival of subjects who did not receive and those who received temozolomide and their GBM tumors expressed high level of IL-13Ra2 mRNA. However, TCGA database did not have enough number of samples from subjects with high IL-13Ra2 gene expression who did not receive temozolomide to perform data analysis for statistical significance. Overall, our findings suggest that IL-13Ra2 over expression is associated with temozolomide resistance in GBM patients.

Expression of IL-13Ra1 and IL-4Ra and their correlations with IL-13Ra2 in GBM

Three different types of IL-13 receptors have been reported [27, 28]. In type I IL-13R, three chains (IL-13Ra1, IL-13Ra2, and IL-4Ra) are present that bind IL-13. Type II IL-13R consists of IL-13Ra1 and IL-4Ra chains. In type III IL-13R, an additional component of IL-2Rγ (common γ chain, γc) is present. We first investigated whether the expression of IL-13 receptor chains (IL-13Ra1, IL-13Ra2, and IL-4Ra) in GBM correlated with each other. Although mRNA for three chains of IL-13 receptor are expressed at different levels in GBM samples, we did not find any correlation between the expression of IL-13Ra2 and of IL-13Ra1 (Fig. 4a) mRNA, nor any correlation between the expression of IL-13Ra2 and of IL-4Ra mRNA (Fig. 4b). In contrast, the expression of IL-13Ra1 mRNA positively correlated with IL-4Ra indicating that these two receptors may form a complex in GBM (Fig. 4c). Furthermore, although low level IL-2Rγ mRNA was also detected, no correlation between the expression of IL-2Rγ (common γ chain, γc) and either the expression of IL-13Ra1, or of IL-13Ra2 was detected, except week correlation with IL-4Ra expression in GBM (Supplementary Fig. S7a-c).
We further investigated the possible correlation between expressions of cytokine IL-13, IL-4 and IL-13 receptors. We found that there was no correlation between expression of IL-13 and of IL-13 receptors (Supplementary Fig. S7d, f) and no correlation between expression of IL-4 and IL-13 receptors (Supplementary Fig. S7e, g). Since IL-4 and IL-13 are predominantly produced by Th2 and other cells but not GBM [29], this observation was not unexpected. Furthermore, the data indicated that there was no possible IL-13 or IL-4 autocrine signaling involved in GBM tumors.

High IL-13Rα1 expression is also associated with poor patient prognosis

To investigate whether IL-13Rα1 mRNA expression is also associated with patient prognosis, Kaplan–Meier survival analysis was performed to assess survival of subjects with high IL-13Rα1 (Log2 ratio > 2) and no IL-13Rα1 mRNA (Log2 ratio < 0) expressing GBM tumors. Subjects with highly expressed IL-13Rα1 mRNA had a median survival of 350 days (n = 65) compared to 693 days for subjects with no IL-13Rα1 mRNA tumors (n = 32) (Fig. 5a). The median survival time between IL-13Rα1 positive and negative groups was highly statistically significant (p < 0.0001).

Although we did not find any correlation between IL-13Rα1 mRNA and IL-13Rα2 mRNA expression, we investigated whether both IL-13Rα1 and IL-13Rα2 expression combined is associated with patient prognosis. Subjects with both IL-13Rα1 and a2 high expression (both Log2 ratio > 2) had a median survival of 337 days (n = 39).
compared to 1256 days for subjects with both IL-13Rα1 and α2 negative tumors (n = 14) (Fig. 5b). The median survival time between both IL-13Rα1 and α2 high and both IL-13Rα1 and α2 negative group was highly statistically significant (p < 0.0001). Subjects with both IL-13Rα1 and α2 high expressions will have poorest survival rate in GBM patient population.

**Verification of gene expression data from microarray technology by RNA-Seq technology**

RNA-Seq provides a far more precise measurement of levels of transcripts than any other methods [30]. A total of 163 RNA-Seq data files were downloaded from TCGA, and only 94 data files overlapped with microarray gene expression files, meaning that only 94 GBM tumors in TCGA have both microarray and RNA-Seq datasets (Fig. 6a). Even though there was only a limited amount of RNA-Seq data available in GBM dataset, we used the RNA-Seq data to evaluate the reliability of our transformed microarray gene expression data. We found that the expression of IL-13Rα2 gene from microarrays showed a strong positive correlation with the counts of reads that align with the IL-13Rα2 gene detected by RNA-Seq technology. IL-13Rα2 expression was strongly correlated (r = 0.898) between the microarray and RNA-Seq data indicating that the transformed Log2 ratio was very reliable, and represented the alterations of genes in GBM compared to the normal brain tissue (Fig. 6b).

**Analyses of immune regulatory genes associated with IL-13Rα2 expression**

In order to investigate the possible connection of immune genes with IL-13Rα2 expression in GBM, we selected the Immport Gene List, which contained 4815 immune-associated genes, from InnateDB (http://www.innatedb.com), which is an integrated analysis platform that has been specifically designed to facilitate systems-level analyses of mammalian innate immunity networks, pathways and genes [31]. We analyzed expression of immune associated genes in IL-13Rα2 highly expressed GBM group and compared with IL-13Rα2 negative GBM group. A total of 132 genes were identified that were overexpressed in IL-13Rα2 highly expressing tumors (Table 1 and Supplementary Table S1).

Among immune associated genes, we looked at the expression of immunosuppressive genes. We found that FMOD (fibromodulin), CCR5 (chemokine receptor), and OSM (oncostatin M) genes were highly expressed in IL-13Rα2 highly expressed group, but not in IL-13Rα2 negative group. FMOD is associated as a risk factor of poor GBM prognosis [32], while CCR5, as a cell surface receptor, plays a role in cancer cell proliferation, metastasis, and the formation of an immunosuppressive microenvironment [33].

Oncostatin M (OSM), a cytokine belonging to the interleukin-6 family, has been shown to be increased in a variety of cancers, including malignant glioma. OSM induces ADM (Adrenomedullin) expression in astroglia cells through induction of signal transducer and activator of transcription-3 (STAT-3) phosphorylation, which is involved in glioma development and progression [34].

CCL2 (chemokine (C-C motif) ligand 2) and CXCL13 (chemokine (C-X-C motif) ligand 13) genes are also highly expressed in the IL-13Rα2 high group. The role of CCL2 and its receptors in the attraction of monocytic myeloid-derived suppressor cells (MDSCs) has been reported. An accumulation of monocytic MDSCs in tumors occurred via an interaction between CCL2 and its receptors. Monocytic MDSCs exhibited CCR2-dependent immunosuppressive activities. The production of CCL2 led to the MDSC accumulation in cancer environment [35].
Table 1 Selected immune related genes expressed only in IL-13Rα2 mRNA highly expressed GBM tumors (Group III)

| Gene symbol | Gene name                                         | Log2 ratio |
|-------------|---------------------------------------------------|------------|
| FMOD        | Fibromodulin                                      | 1.92       |
| CXCL13      | Chemokine (C–X–C motif) ligand 13                 | 1.61       |
| MNDA        | Myeloid cell nuclear differentiation antigen      | 1.58       |
| HLA-DRA     | Major histocompatibility complex, class II, DR alpha | 1.52       |
| TLR8        | Toll-like receptor 8                              | 1.50       |
| LY75        | Lymphocyte antigen 75                             | 1.48       |
| IL15        | Interleukin 15                                    | 1.48       |
| IL18        | Interleukin 18 (interferon-gamma-inducing factor) | 1.45       |
| CCR5        | Chemokine (C–C motif) receptor 5 (gene/pseudogene) | 1.43       |
| CCL2        | Chemokine (C–C motif) ligand 2                    | 1.37       |
| ITGBL1      | Integrin, beta-like 1 (with EGF-like repeat domains) | 1.36       |
| OSM         | Oncostatin M                                      | 1.36       |
| WISP1       | WNT1 inducible signaling pathway protein 1        | 1.35       |
| TREM2       | Triggering receptor expressed on myeloid cells 2  | 1.35       |
| SRGN        | Serglycin                                         | 1.33       |
| GJB6        | Gap junction protein, beta 6, 30 kDa              | 1.33       |
| MMRN1       | Multimerin 1                                      | 1.25       |
| FYB         | FYN binding protein                               | 1.25       |
| PLA2G4A     | Phospholipase A2, group IVA                        | 1.23       |
| IL17B       | Interleukin 17B                                   | 1.23       |
| TLR6        | Toll-like receptor 6                              | 1.23       |
| TLR5        | Toll-like receptor 5                              | 1.23       |
| AIF1        | Allograft inflammatory factor 1                   | 1.21       |
| FOXJ1       | Forkhead box J1                                   | 1.20       |
| TSPO        | Translocator protein (18 kDa)                     | 1.20       |
| GZMK        | Granzyme K (granzyme 3; trypase II)               | 1.20       |
| THBD        | Thrombomodulin                                    | 1.19       |
| SULF1       | Sulfatase 1                                       | 1.18       |
| IFI6        | Interferon, alpha-inducible protein 6             | 1.17       |
| BTG3        | B cell translocation gene 3                       | 1.16       |
| SERPINC1    | Serpin peptidase inhibitor, clade G (C1 inhibitor), member 1 | 1.15 |
| SYK         | Spleen tyrosine kinase                            | 1.14       |
| CSF3R       | Colony stimulating factor 3 receptor (granulocyte) | 1.13       |
| TGFB1       | Transforming growth factor, beta receptor 1       | 1.12       |
| PSMB10      | Proteasome (prosome, macropain) subunit, beta type, 10 | 1.12 |
| SIPA1       | Signal-induced proliferation-associated 1         | 1.11       |
| CIITA       | Class II, major histocompatibility complex, transactivator | 1.11 |
| LILRB1      | Leukocyte immunoglobulin-like receptor, subfamily B 1 | 1.11 |
| TNFRSF14    | Tumor necrosis factor receptor superfamily, member 14 | 1.11 |
| TIPARP      | TCDD-inducible poly(ADP-ribose) polymerase        | 1.10       |
| LCP2        | Lymphocyte cytosolic protein 2                    | 1.10       |
| FPR1        | Formyl peptide receptor 1                         | 1.10       |
| CFD         | Complement factor D (adipsin)                     | 1.10       |
| ATF5        | Activating transcription factor 5                 | 1.10       |
| ANXA4       | Annexin A14                                       | 1.09       |
| LCP1        | Lymphocyte cytosolic protein 1 (t.-plastin)       | 1.09       |
| CFH         | Complement factor H                               | 1.09       |
| OXTR        | Oxytocin receptor                                 | 1.08       |
| IL33        | Interleukin 33                                    | 1.08       |
| CD226       | CD226 molecule                                    | 1.08       |
| F3          | Coagulation factor III (thromboplastin, tissue factor) | 1.07 |
Table 1 (continued)

| Gene symbol | Gene name                                      | Log2 ratio |
|-------------|------------------------------------------------|------------|
| CFLAR       | CASP8 and FADD-like apoptosis regulator         | 1.07       |
| IL7R        | Interleukin 7 receptor                          | 1.03       |
| BCAP29      | B cell receptor-associated protein 29           | 1.03       |
| IFI35       | Interferon-induced protein 35                   | 1.02       |
| MAGED1      | Melanoma antigen family D, 1                   | 1.02       |
| IFT52       | Intraflagellar transport 52 homolog (chlamydomonas) | 1.02 |
| CD276       | CD276 molecule                                  | 1.02       |
| TXAS1       | Thromboxane A synthase 1 (platelet)             | 1.02       |
| CXCR4       | Chemokine (C–C motif) receptor 4                | 1.01       |

*Log2 ratios are the mean values of immune related genes expressed in IL-13Rα2 highly expressed tumor group (Group III)

Discussion

We demonstrate that GBM tumors can be classified into three different distinct groups based on the analysis of gene expression data from 428 glioma subjects at the TCGA database for IL-13R (α1 and α2) gene expression. Group I tumors do not express IL-13Ra1 and α2 mRNA; group II tumors expressed mild to moderate levels of IL-13Ra1 and α2 mRNA, while groups III tumors expressed high level of IL-13Ra1 and α2 mRNA. A large % of GBM samples (76%) expressed mild to moderate levels (Log2 > 0 to < 2) of IL-13Ra1, while 28% expressed mild to moderate levels of IL-13Ra2 mRNA. More than 42% of GBM samples were highly positive for IL-13Ra1 mRNA (Log2 ≥ 2) while only 16% samples were highly positive for IL-13Ra2 mRNA. Patients with group III tumors had the shortest overall survival irrespective of treatment compared to group I and group II patients. Thus overexpression of IL-13Ra1 and α2 gene expression in tumors is associated with decreased patient survival and poor patient prognosis. In addition, patients with highest expression of both IL-13Ra1 and α2 mRNA in tumors showed poorest survival in GBM patients. IL-13Ra2 mRNA expression was confirmed by RNA-seq technology. These results indicate that IL-13R expression in glioma tumors is associated with poor patient prognosis and it is possible that IL-13Rs are prognostic indicator for GBM. Furthermore, when IL-13Ra2 expression was compared between lower-grade glioma and high grade GBM, both IL-13Ra1 and IL-13Ra2 gene expression levels were significantly lower in lower-grade glioma compared with GBM. These results suggest that IL-13Ra2 gene expression may also be associated with GBM malignancy grade.

Temozolomide-based therapy is the standard of care for patients with GBM. However, the efficacy of standard temozolomide chemotherapy and radiation therapy for patients with GBM is compromised by resistance to these therapies. In vitro studies demonstrated that CD133 positive GBM cells show strong tumor’s resistance to chemotherapeutic agents, including TMZ [36]. It was found that expression levels of CD74 in high grade gliomas were inversely associated with TMZ resistance in GBM xenograft lines, suggesting a role in TMZ resistance [37]. In addition, the resistance to TMZ has been shown to be modulated by the DNA repair protein O6-methylguanine-DNA methyltransferase (MGMT). It has been shown that elevated MGMT protein levels or lack of MGMT promoter methylation is associated with TMZ resistance in some, but not all GBM tumors [38]. Although DNA methylation of the MGMT gene promoter was shown to be a prognostic marker for treatment response of temozolomide in GBM [39], Brennan et al. reported that MGMT status distinguishes responders from non-responders to TMZ only among samples classified as classical subtype of GBM (n = 96), but not among other samples classified as proneural, mesenchymal, and neural subtypes of GBM (a total of n = 225) [40]. Their data indicate that MGMT DNA methylation can only be used as a prognostic marker for the classical subtype of GBM, but not for any other subtypes of GBM [40]. In our study, we observed for the first time that the patients with over expressed IL-13Ra2 treated with TMZ chemotherapies had shorter overall survival time compared with the patients with IL-13Ra2 negative expression treated with TMZ, implicating that IL-13Ra2 mRNA expression is associated with GBM resistance to TMZ chemotherapy. In addition, we did not find any correlation of IL-13Ra2 mRNA expression with the MGMT expression (Supplementary Table S2), indicating that the influence of IL-13Ra2 expression on TMZ response was independent of the expression of MGMT. These data suggest that IL-13Ra2 may be a new modulator of TMZ response, representing a distinct mechanism of TMZ resistance from MGMT.

Targeting IL-13Ra2 has motivated the development of highly effective therapies and novel administration strategies. So far, a total of six clinical trials using IL-13-PE in patients with various malignant gliomas have been completed in the United States (https://clinicaltrials.gov). Early clinical trials (Phase I) targeting IL-13Ra2, by IL-13-PE38QQR via CED
in combination with the current standard of care (surgery, radiotherapy, and temozolomide) showed promising safety and efficacy profiles (http://clinicaltrials.gov). A completed phase III randomized clinical trial of convection enhanced delivery (CED) of IL-13-PE38QQR vs. an FDA approved drug carbustine-releasing Gliadel wafers (GW) for recurrent glioblastoma, showed that IL-13-PE was well tolerated, but it did not show superiority over GW in overall survival. Retroactive data analysis of time-to-progression was significantly higher with IL-13-PE compared to GW [23]. However, tumor specimens from the original surgery were not evaluated for the presence of IL-13 receptors in the enrolled patients in this trial. The outcome could potentially be improved if enrolled patients could be limited only to ones with over expressed IL-13Rα2 in tumor tissue. Furthermore, IL-13-PE treatment data in the TCGA dataset showed that IL-13-PE treated patients had a longer median survival (657 days) compared with median survival of the reference patient group without IL-13-PE treatment (384 days) (Supplementary Fig S8). Although the sample size of IL-13-PE treated patients in TCGA was extremely small (n = 6), these preliminary results have significant implications and indicate that targeting of IL-13Ra2 mRNA in GBM treatment holds a promise, specifically for the IL-13Ra2 positive group of patients.

The mechanism of poor survival of patients with GBM tumors expressing high levels of IL-13Ra1 and IL-13Ra2 is not clear. IL-13 and IL-13Ra2 has been shown to be involved in immune evasion and tolerance mechanisms and thus it is possible that high IL-13Ra1 and IL-13Ra2 expression participates in systemic profound immunosuppression seen in GBM patients [41]. In that regard, we found that several immunosuppressive genes were highly expressed in IL-13Ra2 over expressed tumors, but not in IL-13Ra2 negative tumors. These genes included CCL2, which is known to attract MDSCs in cancer microenvironment. MDSCs represent one of the most important players mediating immunosuppression. These cells may not only inhibit an anti-tumor immunity but also directly stimulate tumorigenesis as well as tumor growth and expansion [35]. MDSCs reduce antigen specific CD8 + T cell proliferation, increase T-cell death by apoptosis, and change the profile of cytokines secreted by activated T lymphocytes [42]. It is possible that targeting MDSCs will have a favorable outcome in patients with GBM. A better understanding of the contribution of the tumor on systemic immune suppression is necessary for improved therapies, to monitor negative effects of novel treatments, and to improve patient outcomes.

Recently, various gene expression studies have identified gene signatures that are associated with various types of GBM as well as signatures that correlate with survival. Kim et al. identified 42 probe sets that show an association with tumor aggressiveness and patient survival [5]. Verhaak et al. identified a gene signature associated with four subtypes (proneural, neural, classical, and mesenchymal) of GBM. An 840 gene signature (210 genes per class) was established. Each of the signatures was highly distinctive [4]. These defined subtypes differ by the type of genetic abnormalities they carried and by the patient’s clinical characteristics. A high level of EGFR expression and EGFR amplification were mainly observed in the classical subtypes. The IDH1 and TP53 mutations were significantly frequent events in the proneural subtype. IDH1 somatic mutation has been linked to a glioma-CpG island methylator phenotype (G-CIMP) [43]. Turcan et al., have demonstrated that IDH1 mutation is the cause of CIMP and leads to CIMP phenotype, and is sufficient to establish the glioma hypermethylator phenotype [44]. G-CIMP tumors belong to the proneural subgroup in GBM and are more prevalent among lower-grade gliomas [43, 45]. In addition, PDGFRA was another gene, which was mutated and highly expressed only in the proneural subtype [4]. Brown et al. reported that high IL-13Ra2 gene expression is positively correlated with the mesenchymal signature gene expression and negatively correlated with the proneural signature gene expression [24]. They further showed that IL-13Ra2 expression is correlated, but not limited to the expression of mesenchymal signature genes. In our study, we did not find any correlation between IL-13Ra2 mRNA expression and previously reported biomarkers of GBM subtypes such as IDH1, EGFR, MGMT, and PDGFRA (Supplementary Table S2). Furthermore, we also found that high expression of IL-13Ra1 and a2 genes are associated with poor prognosis in IDH1-Wt/non-G-CIMP GBM (Supplementary Fig S9a and b). Our Data shows that approximately 70% GBM tumors express moderate to high level of IL-13Ra2 mRNA, which is expressed in most of the subtypes identified by Verhaak et al. [4]. Our recent studies in animals confirm our observations that IL-13Ra2 is involved in tumor invasion, metastasis and poor survival of animals implanted with human pancreatic and ovarian cancers [9, 10]. These results indicate that a single gene (IL-13Ra2) may provide a stronger correlation with survival than a group of genes previously identified, thus making IL-13Ra2 an important target for glioma therapy.

It is of interest to note that mRNA for three chains of IL-13 receptor (IL-13Ra2, IL-13Ra1 and IL-4Ra) are expressed at different levels in GBM samples. IL-13Ra2 was highly expressed in more than 42% of total GBM samples, compare with that of IL-13Ra1 only highly expressed in less than 16% of the total GBM. We did not find any correlation between the expression of IL-13Ra2 and IL-13Ra1, nor between IL-13Ra2 and IL-4Ra mRNA. These analyses indicate that over expression of IL-13Ra2 mRNA in GBM is independent from expression of IL-13Ra1 or IL-4Ra suggesting that IL-13Ra2 does not seem to form a complex with either IL-13Ra1 or IL-4Ra chain. This is consistent with our previous work that summarized by Suzuki et al. and
Joshi et al. [29, 46]. In contrast, the expression of IL-13Rα1 mRNA positively correlated with IL-4Rα indicates that these two receptor chains form a type II IL-13R complex in GBM. Indeed, this receptor has been shown to mediate IL-13 signaling in cancer cells [7, 10, 29].

In conclusion, we have found that high IL-13Rα1 and IL-13Rα2 mRNA expression is associated with poor patient prognosis, demonstrating an inverse relationship between IL-13R expression and overall survival. The similar inverse relationship seems to be also associated with days to GBM recurrence and long-term patient survival. Furthermore, we show for the first time that IL-13Rα2 expression is associated with GBM resistance to TMZ chemotherapy. These findings have important implications in understanding a possible role of IL-13R in GBM pathogenesis, development of targeted therapies, and define a patient population for immunotherapy or alternative therapies in clinical trials. Additional studies are ongoing to further confirm our observations.

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Compliance with ethical standards

Conflict of interest The authors received no specific funding for this work. Both authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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