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Multicellular Network-Informed Survival Model for Identification of Drug Targets of Gliomas

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Abstract

Increasing evidence suggests that communication between tumor cells (TCs) and tumor-associated macrophages (TAMs) plays a substantial role in promoting progression of low-grade gliomas (LGG). Hence, it is becoming critical to model TAM-TC interplay and interrogate how the crosstalk affects prognosis of LGG patients. This article proposed a translational research pipeline to construct the multicellular interaction gene network (MIGN) for identification of druggable targets to develop novel therapeutic strategies. Firstly, we selected immunotherapy-related feature genes (IFGs) for TAMs and TCs using RNA-seq data of glioma mice from preclinical trials. After translating the IFGs to human genome, we constructed TAM- and TC- associated networks separately, using a training set of 524 human LGGs. Subsequently, clustering analysis was performed within each network, and the concordance measure K-index was adopted to correlate gene clusters with patient survival. The MIGN was built by combining the clusters highly associated with survival in TAM- and TC-associated networks. We then developed a MIGN-based survival model to identify prognostic signatures comprised of ligands, receptors and hub genes. An independent cohort of 172 human LGG samples was leveraged to validate predictive accuracy of the signature. The areas under time-dependent ROC curves were 0.881, 0.867, and 0.839 with respect to 1-year, 3-year, and 5-year survival rates respectively in the validation set. Furthermore, literature survey was conducted on the signature genes, and potential clinical responses to targeted drugs were evaluated for LGG patients, further highlighting potential utilities of the MIGN signature to develop novel immunotherapies to extend survival of LGG patients.

Index Terms—

Immunotherapy; multicellular interaction gene network (MIGN); prognostic signature; survival analysis

I. Introduction

Diffuse gliomas represent a heterogeneous collection of aggressive brain tumors. According to World Health Organization (WHO), diffuse gliomas are classified as grade II through grade IV, indicating different status and malignant potential [1]. As the most notorious tumors, even the low-grade glioma (LGG) (grade II/III) induces profound disability and high mortality [2]. Additionally, more than half of LGGs evolve and progress to grade IV glioma (glioblastoma multiforme, GBM) [3], which has an inferior prognosis with the median survival being only 14 months [4]. Current studies for the classification and prognosis prediction of gliomas mainly focus on GBM, while effective biomarkers for LGG patient prognosis are still limited [5]. Therefore, it is important to detect prognostic signatures for stratifying LGG patients into high-risk or low-risk subgroups and optimizing therapeutic strategies. Moreover, it is desirable to identify high-impact genes that can serve as druggable targets to develop novel immunotherapies for patients not responsive (or resistant) to the current treatments.

Deciphering cell-cell interaction (CCI) provides great opportunities to develop effective cancer immunotherapies [6], [7]. Specifically, targeting tumor-associated macrophages (TAMs) in gliomas represents an attractive therapeutic approach [8], [9]. It has been demonstrated that inhibition of colony-stimulating factor-1 receptor (CSF-1R), the targeted therapy that reprograms TAMs to become antitumorigenic by downregulating M2-like genes, is capable of blocking glioma progression and extending survival in mouse models [10], [11]. However, early-phase clinical trials evaluating CSF-1R inhibition for glioma patients showed little evidence of efficacy [12], [13]. Ni et al. demonstrated that in PTEN-deficient gliomas, Gal-9/Tim-3 expression was elevated, resulting in TAM recruitment and M2 polarization, which facilitated tumor progression. They blocked Tim-3 expression in TAMs, which was shown to attenuate angiogenesis and suppress tumor growth in mouse models of PTEN-null gliomas [14]. Nevertheless, those findings are done by functional experiments, which are time-consuming and limited to single therapeutic target per experiment. In order to interrogate the genome-wide crosstalk between TAMs and tumor cells (TCs) for identifying druggable genes associated with survival, there are multiple practical challenges in study design considerations. The first challenge comes from a general lack of large-sample data with both clinical information and cell type-specific gene expression of glioma patients. The second one lies in the complex modelling of CCI networks connecting TAMs and TCs. Additionally, effective identification of signature genes that possess druggable potential and predictive value remains challenging.

Here, we proposed a translational research pipeline to construct the multicellular interaction gene network (MIGN), systematically investigate TAM-TC communication, and effectively identify drug targets for the development of novel immunotherapies. Firstly, RNA-seq data from preclinical trials that treated glioma mice with CSF-1R inhibition [11] were employed to screen immunotherapy-related feature genes (IFGs) for both TAMs and TCs. After translating the IFGs to human genome, we constructed TAM- and TC- associated networks separately, using a large LGG cohort from The Cancer Genome Atlas (TCGA) database. Then clustering analysis was conducted within each network, and the concordance measure K-index was adopted to correlate gene clusters with patient survival. Those clusters that

highly correlated with survival in each TAM- and TC- associated network were combined to build the MIGN, in which TAM/TC clusters were merged if their underlying pathways worked together to co-influence disease progression. The cluster-derived hub genes and potentially activated ligands and receptors were identified in the MIGN to constitute prognostic signatures for survival prediction and therapeutic intervention. Subsequently, an independent LGG cohort from the Chinese Glioma Genome Atlas (CGGA) database was adopted to validate the predictive power of the MIGN signature. Additionally, single-cell RNA-seq data for LGG patients was used to verify that our signature genes were involved in the regulation of TAM-TC interplay. Finally, the potential sensitivity to targeted drugs was assessed based on the MIGN signature using an independently developed bioinformatics pipeline.

II. Methods

A. IFG Selection From Preclinical Mouse Trials

The flowchart of the translational pipeline to construct the multicellular interaction gene network (MIGN) and identify drug targets of immunotherapies is presented in Fig. 1. We began with experimental results of the preclinical mouse study that investigated long-term treatment efficacy of CSF-1R inhibition in gliomas [11]. After downloading the preclinical RNA-seq data from the Gene Expression Omnibus (GEO) website (<https://www.ncbi.nlm.nih.gov/geo/>) with accession number GSE69104, we selected mouse samples that contained cell type-specific gene expression for both tumor-associated macrophages (TAMs) and tumor cells (TCs), leading to four drug-resistant samples, six drug-sensitive samples, and five vehicle (non-treated) samples.

For both TAMs and TCs, we focused on selecting immunotherapy-related feature genes (IFGs) that expressed differentially between drug-resistant and drug-sensitive samples during CSF-1R inhibition. First, we normalized the expression level of each gene in the treated groups (drug-resistant / drug-sensitive) to its mean level in the non-treated group as follows: $z_{ij} = \log_2(x_{ij}) / \log_2(\bar{v}_i)$, where x_{ij} is the expression value of the i -th gene in j -th sample of the treated groups, and $\bar{v}_i$ is the mean expression of the i -th gene across samples in the non-treated group. Next, we compared normalized gene expression profiles between drug-resistant and drug-sensitive samples using t-test and fold change (FC). Those genes with false discovery rate (FDR)-adjusted p -value < 0.05 in t-test and $|FC| > 1.5$ were selected as IFGs, which were then translated to those in human genome by the online tool (NCBI, <https://www.ncbi.nlm.nih.gov>) for further analysis.

To construct the weighted gene correlation network and perform survival analysis, we collected clinical information and RNA-seq gene expression data for LGG patients in TCGA database (<https://cancergenome.nih.gov/>) and CGGA database (<http://www.cgga.org.cn/>). After matching the clinical information and RNA-seq data for each sample, one cohort of 524 LGG patients from the TCGA database was prepared for training, and an independent cohort of 172 LGG patients from the CGGA database was prepared for validation.

B. Construction of TAM- and TC- associated Gene Networks

Next, the TCGA dataset was leveraged to construct TAM- and TC- associated gene networks with the translated IFGs for TAMs and TCs being their node sets, respectively. Generally, weighted gene correlation network was denoted as $G = (V, W)$, where V was the node set, and $W = (w_{ij})$ was a $|V| \times |V|$ positively weighted adjacency matrix. Let x_i and x_j denote the expression levels of the i -th and j -th gene across all the samples, the entry w_{ij} was encoded as the power function of the absolute value of Pearson correlation coefficient:

$$w_{ij} = w_{ij}(\beta) = |\text{cor}(x_i, x_j)|^\beta, i \neq j, \quad (1)$$

where β was referred to as the soft threshold parameter. Since the correlation between each gene and itself was not considered, the diagonal elements of W were set to 0. Then we could define the connectivity degree for the i -th gene:

$$k_i(\beta) = \sum_{j=1}^{|V|} w_{ij}(\beta), i = 1, 2, \dots, |V| \quad (2)$$

and the connectivity frequency for the network:

$$f(k) = \sum_{i=1}^{|V|} I(k_i(\beta) \in (k-1, k]), k = 1, 2, 3, \dots \quad (3)$$

We assume that gene network is dominated by a few highly connected nodes. Thus, we selected soft threshold parameter β to ensure the network satisfying scale-free topology criteria [15], [16], which are characterized by power law distribution of the connectivity proportion, i.e.,

$$p(k) \sim k^{-\gamma}, \quad (4)$$

where $p(k) = f(k)/|V|$, and γ is a positive constant. After taking logarithm on both sides of (4), the log-proportion of connectivity $\log(p(k))$ is linearly correlated with $\log(k)$. Then linear regression model fitting index R^2 could be used to quantify how well a network satisfies the scale-free criteria.

C. Gene Clustering Analysis Within the Network

Gene clustering analysis was conducted within each of the TAM- and TC- associated networks. Here, we used the hierarchical clustering method in conjunction with topological overlap matrix (TOM)-based dissimilarity to detect gene clusters within the network, as

performed in the R package WGCNA [17]. In specific, the topological overlap matrix $S = (s_{ij})$ is defined based on the weighted adjacency matrix $W = (w_{ij})$ as follows

$$s_{ij} = \frac{l_{ij} + w_{ij}}{\min\{k_i, k_j\} + 1 - w_{ij}}, \quad (5)$$

where $l_{ij} = \sum_{u=1}^{|V|} w_{iu}w_{uj}$, and $k_i = \sum_{u=1}^{|V|} w_{iu}$. And the TOM-based dissimilarity d_{ij} is defined as

$$d_{ij} = 1 - s_{ij}. \quad (6)$$

The dissimilarity d_{ij} summarizes the global connection information between the i -th and j -th genes within the gene network instead of only focusing on their pairwise relationship. Thus, d_{ij} leads to more distinct gene cluster than the standard measure (e.g., 1 minus the absolute value of correlation coefficient) [18], [19]. With the TOM-based dissimilarity, we obtained a clustering dendrogram using the average linkage hierarchical clustering method. The dynamic tree cut method [20] was adopted to cut branches off the dendrogram, and thus we obtained several distinct gene clusters within the network. In the R package WGCNA, the parameter `deepSplit` controls how finely the branches should be split. The higher value of `deepSplit` gives smaller clusters. Another parameter `minClusterSize` limits the minimum number of genes in each cluster.

D. MIGN Construction for Exploration of Communication Between TAMs and TCs

Within the TAM- and TC- associated networks, the concordance measure K-index [21] was adopted to correlate gene clusters with patient survival. Typically, gene clusters were well represented by their eigengenes (i.e., the first principal component of the expression matrix of the gene cluster). Thus, for TAM- and TC- associated networks, the eigengene-based K-index was calculated for each cluster as

$$K - index = \frac{2}{n(n-1)} \sum_{i \neq j} \frac{I(R_j > R_i)}{1 + \exp(R_j - R_i)}, \quad (7)$$

where n denotes the sample size of involved patients, and R_i indicates the eigengene-based risk score for the i -th patient. Consequently, gene clusters with $K - index > 0.6$ were considered to be highly correlated with survival outcomes in TAM- and TC- associated networks.

To build the multicellular interaction gene network (MIGN), we combined genes in those clusters that identified to be highly correlated with patient survival in TAM- and TC- associated networks. Then scale-free network was constructed with the selected genes, and clustering analysis was performed within the network. Given the intense signaling

interaction between TAMs and TCs during glioma development, the selected TAM- and TC-clusters were assumed to merge in the MIGN if their underlying pathways worked together to co-influence disease progression. Fisher's exact test was applied to examine the correspondence of gene clusters between MIGN and TAM/TC-associated networks.

E. Identification of the MIGN Prognostic Signature

Cell-cell communication was mainly mediated by secreted ligands and corresponding cell-surface receptors. We collected prior information on ligand and receptor genes from the Omni-path database [22], and screened TAM ligands and TC receptors in the TAM/TC interaction gene clusters of MIGN. Besides, hub genes were selected as those with $K - index > 0.6$ and connectivity $k(\beta)$ within the top 10% of each gene cluster in the MIGN. With the selected ligands, receptors, and hub genes, we conducted LASSO-based Cox regression on each type of candidate genes to shrink the feature size. Next, a stepwise variable selection procedure (with iterations between the 'forward' and 'backward' steps) for Cox regression was applied to identify significant features. Patient age and tumor grade were considered when selecting variables to achieve a more accurate prediction. Consequently, we constructed the MIGN signature by fitting multivariate Cox regression model using the selected signatures

F. Evaluation Measures of the MIGN Signature

Log-rank test and time-dependent ROC were used to evaluate prediction performance of the MIGN signature. For gene expression profile X in both training and validation datasets, the risk score $f(X) = X'\hat{\beta}$ was computed, where $\hat{\beta}$ denoted regression coefficients derived from the multivariate Cox regression model. Patients were classified into high-risk or low-risk groups by choosing the median of risk scores as cutoff in each dataset, indicating poor or good prognoses. Kaplan-Meier (K-M) curves were then generated to summarize patient survival in each risk group, and log-rank test was conducted to assess whether survival curves for high-risk and low-risk groups were significantly different. Time-dependent ROC analysis was performed to evaluate accuracy of the MIGN signature in predicting 1-year, 3-year, and 5-year survival rates. Larger value of the area under the ROC curve (AUC) indicates better prediction of patient survival.

G. Dissecting TAM-TC Interactions in LGG By Multilayer Signaling Network Analysis

We collected single-cell RNA sequencing (scRNA-seq) data for LGG patients from the publicly available database Gene Expression Omnibus (GEO) (www.ncbi.nlm.nih.gov/geo/) with accession number GSE182109 to verify the crosstalk between TAMs and TCs. Seurat v4.0.3 was used to perform quality control (QC), normalization, feature selection, and dimensional reduction for the scRNA-seq data. Low-quality cells containing <500 expressed genes or >20% mitochondrial transcripts or >5% ribosomal transcripts were removed. Additionally, principal components were batch-corrected for variations between tumor specimens using the Harmony R package. Potential single-cell doublets were identified using the DoubletFinder R package. Uniform Manifold Approximation and Projection (UMAP) was used for visualization of the scRNA-seq data. Moreover, multilayer signaling

network analysis was performed to infer the intercellular communication between TAMs and TCs using scMLnet [23], an R package developed by our group previously.

H. Assessing Potential Drug Sensitivity to New Treatments Based on the MIGN Signature

Since the translational pipeline was originated from a mouse study that proposed IGF-1R inhibition as the treatment to drug resistance developed during CSF-1R blockade, it is of interest to investigate the potential drug sensitivity of human LGG patients to the related therapeutics. As an independently-developed bioinformatics pipeline, the oncoPredict R package [24] could predict IC50 (the half maximal inhibitory concentration) of a large number of drugs screened in cancer cell lines based on gene expression data of cancer patients. Thus, we calculated potential clinical responses (IC50) to targeted drugs for LGG patients using the oncoPredict package, with the authoritative drug response resource Genomics of Drug Sensitivity in Cancer (GDSC) [25] being the training set. We attempted to uncover treatments that exhibit significantly different sensitivity scores between the high-risk and low-risk groups of patients stratified by the MIGN signature, which may bring clinical benefit from the perspective of precision medicine.

III. Results

A. MIGN Construction and Analysis

We proposed a translational pipeline to construct the multicellular interaction gene network (MIGN), to model communication between macrophages and glioma cells for LGG patients, and to facilitate genome-wide screening of new drug targets for immunotherapies. As demonstrated in the flowchart (Fig. 1), we first selected immunotherapy-related feature genes (IFGs) for TAMs and TCs using cell-type specific RNA-seq data of existing randomized preclinical mouse trials [11]. After translating them to human genome, we obtained 255 IFGs for TAMs and 795 IFGs for TCs, respectively. Prior to building the MIGN, we constructed and analyzed TAM- and TC- associated networks (see section Methods for details).

For the TAM-associated network $G_1 = (V_1, W_1)$, the vertex set V_1 contained 255 nodes that being the TAM-specific IFGs, and weighted adjacency matrix W_1 was built by setting the soft threshold parameter β to 5. Thus, the network approximately satisfied scale-free topology criteria with the index R^2 reaching 0.97. By setting the clustering parameters deepSplit as 3 and minClusterSize as 25, four gene clusters were identified in the TAM-associated network (Fig. 2(a)). Two clusters (TAM-brown and TAM-turquoise) were considered being highly associated with overall survival, with K-index for their eigengenes greater than 0.6 and their K-index distributions were significantly higher than those of others (Fig. 2(c)).

For the TC-associated network $G_2 = (V_2, W_2)$, the vertex set V_2 contained 795 nodes that being the TC-specific IFGs, and weighted adjacency matrix W_2 was built by setting the soft threshold parameter β to 5. Thus, the network approximately satisfied scale-free criteria with the index R^2 reaching 0.84. By setting the clustering parameters deepSplit as 3 and minClusterSize as 30, six gene clusters were identified in the TC-associated network (Fig.

2(b)). Three clusters (TC-blue, TC-green and TC-turquoise) were considered being highly associated with overall survival, with K-index for their eigengenes greater than 0.6 and their K-index distributions were significantly higher than those of others (Fig. 2(d)).

To construct the MIGN $G_3 = (V_3, W_3)$, we combined genes in those five clusters (TAM-brown, TAM-turquoise, TC-blue, TC-turquoise, and TC-green) that identified to be highly correlated with patient survival in TAM- and TC- associated networks. Here, the vertex set V_3 contained a total of 609 nodes, and weighted adjacency matrix W_3 was built by setting the soft threshold parameter β to 5, making the network approximately satisfied scale-free topology criteria with the index R^2 greater than 0.8 (Fig. 3(a)). With the deepSplit set to 3 and minClusterSize set to 30, four gene clusters (MIGN-blue, MIGN-brown, MIGN-turquoise, and MIGN-grey) were identified in MIGN (Fig. 3(b)). All the clusters other than MIGN-grey were highly correlated with patient survival (K-index > 0.6), and their K-index distribution was significantly higher than that of MIGN-grey (Fig. 3(c)). Additionally, there was a significantly positive correlation between K-index and connectivity in these three clusters (Fig. 3(d)), allowing us to select hub genes in subsequent analysis.

B. MIGN Signature Identification for LGG Patients

Next, contingency tables were generated to examine the correspondence of gene clusters between MIGN and TAM/TC- associated networks (Fig. 4(a) and (b)). Consequently, the clusters MIGN-blue and MIGN-brown that comprised of both TAM- and TC-clusters were referred to as the interaction gene clusters (IGCs), and the cluster MIGN-turquoise that contained single type of cell-related genes was a non-interaction gene cluster (non-IGC). Then KEGG enrichment analysis was performed on each of the gene clusters to explore their biological implications (Fig. 4(c)). Both the IGCs (MIGN-blue and MIGN-brown) were enriched in cell adhesion molecules. Despite involved in cell adhesion pathways, MIGN brown was also enriched in several cancer-related pathways (e.g., TNF signaling pathway). We screened TAM ligands and TC receptors in the IGCs since these clusters represent intercellular signaling communication between TAMs and TCs. Besides, hub genes were selected as those with $K - index > 0.6$ and connectivity $k(\beta)$ within the top 10% of both the IGCs and non-IGC.

We first applied LASSO-based Cox regression on each type of the candidate genes (ligands, receptors, and hub genes) to shrink the feature size. Then we applied stepwise Cox regression to identify the significant features, in which significance levels for entry (SLE) and stay (SLS) were set at 0.15 to be conservative. Patient age and tumor grade were considered when selecting variables to achieve a more accurate prediction. Additionally, the significant TAM ligands (Fig. 5) were always included in the model, though they may lose significance due to the masking effects of intermediate variables when combined with receptors and hub genes. As a result, we obtained the MIGN signature that comprised of four ligands (EFNB2, C3, IGF1, TNF), four receptors (LRRC4, FXVD6, GFRA1, TNFRSF21), seven hub genes (ASPM, SOX6, ACVR2B, ATCAY, MSR1, PAK7, DUSP26), and two clinical factors (Age and Grade). The forest plot together with the coefficient, p-value and hazard ratio of the MIGN signature in the multivariate Cox regression model were presented in Fig. 6.

C. Performance Evaluation, Comparison, and Functional Investigation of the MIGN Signature

We performed K-M and time-dependent ROC analyses to evaluate the performance of MIGN signature with respect to predicting patient survival. For both the training and validation datasets, the risk score was calculated for LGG patients to rank the sample in descending order. We assigned the top 50% of patients as the high-risk group and the bottom 50% as the low-risk group according to the median risk scores. K-M curves for those two groups of patients were well separated and the difference was significant according to the log-rank test in both the training and validation datasets (Fig. 7(a) and (b)). Time-dependent ROC analysis was then performed to assess prognostic accuracy. For the training dataset, the time-dependent AUCs with respect to 1-year, 3-year, and 5-year survival rates were 0.923, 0.902, and 0.83, respectively (Fig. 7(c)). For the validation dataset, the time-dependent AUCs with respect to 1-year, 3-year, and 5-year survival rates were 0.881, 0.867, and 0.839, respectively (Fig. 7(d)). We tested prognostic significance of the MIGN signature using a bootstrapping approach. We randomly selected 15-gene sets from the whole transcriptome 1000 times and compared their predictive accuracy with that of the MIGN signature. The ROC curves of the MIGN signature against bootstrapped 15-gene signatures for the validation set of CGGA cohort justified the prognostic significance of the MIGN signature Fig. 7(e). These results suggest that the MIGN signature has good prognostic significance and accuracy in both the training and validation datasets.

Moreover, we compared the predictive accuracy of the MIGN signature with three other risk signatures designed for glioma patients. For clarity, the MIGN signature proposed in this study is denoted as signature 1; signature 2 denotes the macrophage-related gene signature proposed by Sun et al. [26] based on 12 genes (ANPEP, DPP4, PRRG1, GPNMB, TMEM26, PXDN, CDH6, SCN3A, SEMA6B, CCDC37, FANCA, and NETO2); signature 3 represents the immune-related gene signature proposed by Zhang et al. [27] with 6 genes (CANX, HSPA1B, KLRC2, PSMC6, RFXAP, TAP1); signature 4 denotes the clinical factor signature (Age and Grade). To achieve a fair comparison, we adjusted signature 2 and signature 3 with the clinical factors (Age and Grade) and re-trained their coefficients using the TCGA dataset, which was exactly the training set for the MIGN signature. Consequently, the time-dependent ROCs for the validation set of CGGA cohort indicated that the MIGN signature outperformed all the other risk signatures (Fig. 8(a)–(c)).

To investigate biological functions of the MIGN signature, we conducted a literature survey on the associated genes. As demonstrated in Fig. 8(d), all the signature genes were engaged in biological processes of tumor growth and metastasis or have been reportedly correlated with neuronal function. For example, IGF1 is known to regulate proliferation and migration of glioma cells, and IGF1 together with its receptor IGF1-R appears to trigger LGG progression to GBM [28]. Thus, the IGF1 signaling may provide therapeutic opportunities for LGG patients. In addition, some of our signature genes have been validated as druggable targets in glioma studies (Fig. 8(d)), further highlighting potential utilities of the MIGN signature to develop novel treatments to extend survival of LGG patients.

To further explore the prognostic value of the MIGN signature in stratified cohorts, we classified LGG patients in the TCGA dataset according to their treatment status and assessed

the survival difference between high-risk and low-risk patients within each treatment group. Consequently, the MIGN signature retained prognostic significance for LGG patients treated with or without pharmaceutical therapy (Fig. 9(a) and (b)) and radiotherapy (Fig. 9(c) and (d)), indicating that the MIGN signature could accurately identify patients with an unfavorable prognosis regardless of their treatment characteristics. Additionally, we looked into IDH1, IDH2 and TP53 mutation status with the information available from CGGA dataset. It appears that there is a significant difference between risk scores evaluated by MIGN signature of mutant and wild-type groups of patients (Fig. 9(e)), indicating the association between MIGN signature and genomic alterations in low-grade gliomas.

D. Verification of TAM-TC Interactions in LGG Using scRNA-Seq

To provide further mechanistic evidence on the interplay between TAMs and TCs in LGG patients, we leveraged the scRNA-seq data of human LGG tissues [29] to infer cell-cell interactions in the tumor microenvironment. After cell filtration based on QC metrics, we obtained 25986 cells from 4 tissues for subsequent analysis. PCA was applied to reduce the dimensionality of data, and the top 2000 most variable genes were used for cell clustering (Fig. 10(a)). The computed PCs were batch-corrected for variations between tumor specimens using the Harmony R package (Fig. 10(b)), and doublet cells were removed using the DoubletFinder R package (Fig. 10(c)). After re-clustering the remaining single cells, we obtained nine cell clusters with distinct gene expression patterns (Fig. 10(d)). Using the marker genes specifically expressed in each cluster, we classified cell clusters as either macrophage (CD14, AIF1, FCER1G, TYROBP, CSF1R), glioma cells (PDGFRA, SOX2, NES, GFAP, EGFR), oligodendrocytes (MBP, TF, MAG, MOG, CLDN11), stromal cell (PDGFRB, ACTA2, PROM1, PECAM1, TEK) or T cells (CD2, CD3D, CD3E, CD3G) (Figs. 10(e) and 11(a)).

Cell-cell communication networks globally depicted the intercellular ligand-receptor interactions between the above cell types, which indicated that TAM-TC interactions were among the most abundant cell-cell communication in the tumor microenvironment (Fig. 11(b)). Moreover, the MIGN signature-derived ligands and receptors (IGF1, EFNB2, TNF, TNFRSF21, LRRC4) were verified to mediate TAM-TC interactions as shown in the multilayer signaling network (Fig. 11(c)), where the downstream TFs (e.g., EGR1, MYC, and TP53) activated target genes related to PI3K signaling pathway in glioma cells [30]. Collectively, these results indicated that the MIGN signature plays a vital role in regulating intercellular communication between TAMs and TCs, and it thus implicates opportunities to block LGG progression by biological interventions targeting TAMs.

E. Independent Evidence of Drug Sensitivity to IGF-1R Inhibition Based on the MIGN Signature

Potential clinical responses of LGG patients to target therapies were calculated by the oncoPredict R package [24]. Genomics of drug sensitivity in cancer (GDSC), an authoritative resource for drug response data, was used as the training set. Using gene expression data of LGG patients in the TCGA dataset, we found three IGF-1R inhibitors (Linsitinib, NVP-ADW742, and GSK1904529A) that exhibited significantly different responses between high-risk and low-risk groups classified by the MIGN signature (Fig.

12(a)). Besides, those IGF-1R inhibitors displayed markedly discriminative sensitivity scores for high-risk and low-risk groups in the CGGA cohort (Fig. 12(b)). For both the TCGA and CGGA cohorts, the low-risk group consistently presented lower IC50 compared to the high-risk group, indicating that patients in the low-risk group were more sensitive to the IGF-1R inhibitors and may thus benefit a better therapeutic effect from this treatment.

On the other hand, gliomas are characterized by substantial heterogeneity among tumors, underscored by the identification of several molecular subtypes (classical, mesenchymal, neural, and proneural) [31]. Since proneural gliomas are always associated with better survival than other subtypes [32], [33], we compared sensitivity scores among molecular subtypes of LGG patients to examine whether the proneural patients possess potentially more backup resources for the IGF-1R inhibition. As LGG patients are all missing information on molecular subtypes in the TCGA database, the drug sensitivity exploration was hence focused on LGG patients from the CGGA database. For the IGF-1R inhibitor NVP-ADW742, with a relatively lower IC50 than two other inhibitors (Linsitinib and GSK1904529A) in both the TCGA and CGGA cohorts (Fig. 12(a) and (b)), the predicted value of IC50 for proneural patients was significantly lower than those of other subtypes (Fig. 12(c)). Furthermore, correspondence analysis was conducted to reveal that the proneural patients were highly enriched in the low-risk group classified by the MIGN-based signature (Fig. 12(d)). Taken together, these results support our hypothesis that IGF-1 signaling, the critical pathway that connecting TAM and TC, might provide useful targets for developing novel therapies in human gliomas.

IV. Discussion

Immunotherapies have improved outcomes for many cancer patients, although non-responders still exist widely, and drug resistance develops very quickly. Thus, there is an urgent need to develop novel therapeutic strategies for patients not responsive (or resistant) to the current treatments. Drug development process is typically involved in translating preclinical findings of animal experiments into human patient studies, however, a translational pipeline to facilitate genome-wide screening of new drug targets for developing immunotherapies has not been previously developed. Here, we proposed a gene network-based translational pipeline for identification of drug targets for clinical intervention in low-grade gliomas (LGG). We aimed to identify biologically impactful signature genes that possess therapeutic potential and prognostic value, since patient survival is an important population level surrogate for checking effectiveness of drug targets for advanced cancers such as gliomas.

Biologically, cell-cell interaction (CCI) is an essential part to be considered in developing effective immunotherapies. To model the CCI between tumor cells (TCs) and tumor-associated macrophages (TAMs) for LGG patients, we first selected immunotherapy-related feature genes (IFGs) for TAMs and TCs from an existing animal study. Since treatments in animal models have altered the IFG expression levels, those genes have the potential to be similarly modified in humans, thus improving the likelihood of eventual success in developing novel therapies based on the selected genes. On the other hand, those IFGs are significantly associated with survival outcomes of mice bearing gliomas; they thus may

contain driver genes related to disease progression that are highly predictive of survival. After translating IFGs to those in human genome, we constructed TAM- and TC- associated networks separately, using a large publicly available dataset for LGG patients. Since gene clusters are more likely to be reproducible and often carry high impact than a single gene, those clusters highly associated with patient survival in each TAM- and TC- associated network were selected and combined to build the multicellular interaction gene network (MIGN).

Of note, for the selected clusters in TAM- and TC- associated networks, the connectivity $k(\beta)$ of pivotal genes is positively correlated with patient survival as measured using concordance K-index. This is consistent with our hypothesis that evolutionally-conserved (between mouse and human) genes and gene networks are likely to play essential roles in survival prediction, and genes with high K-index and high connectivity in sub-networks tend to be biologically more impactful. Then cluster-derived hub genes and potentially activated ligands and receptors were identified in the MIGN to constitute the candidate features. For easy biological investigation in lab studies and medical intervention in clinical trials, it is desired to identify a relatively small set of signature genes as therapeutic targets to develop novel cancer treatments. Thus, we implemented LASSO-based and stepwise Cox regression models on the candidate features, obtaining the MIGN signature as a predictor of patient prognosis as well as candidate targets for developing novel immunotherapies. Additionally, scRNA-seq data of human LGG tissues was leveraged to elucidate how TAMs interact with TCs in LGG patients. Multilayer network analysis was adopted to verify that the selected ligands and receptors were involved in mediating macrophage-glioma interactions.

The first limitation of the translational research pipeline proposed in this study is that we have to find proper preclinical or trials for the selection of IFGs. Secondly, the bulk RNA-seq data we used to construct gene networks is the averaged expression profile of the selected IFGs, instead of the cell-type specific expression levels. Furthermore, intercellular signaling transduction is spatially heterogeneous in the tumor microenvironment. Thus, future work may focus on integrating scRNA-seq and spatial transcriptome RNA sequencing to develop novel methodologies to infer cell-cell communication between immune cells and tumor cells and uncover druggable target genes for novel biological interventions.

V. Conclusion

In summary, we have developed a translational pipeline to borrow strength from animal models, and to decipher the genome-wide crosstalk between TAMs and TCs in LGGs. The newly developed strategy addresses critical challenges in interrogating how TAM-TC communication affects patient prognosis, thus it facilitates the identification of signature genes for survival prediction and therapeutic targets for immunotherapy development. Furthermore, potential clinical responses to targeted drugs were evaluated for LGG patients, which uncovered IGF-1R inhibition as the treatment to benefit the low-risk group stratified by the MIGN signature. There is evidence that proneural glioma patients are enriched in the low-risk groups which warrants further investigation and might have implications for new trial design or improve disease management from the perspective of precision medicine.

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Availability of Data and Materials

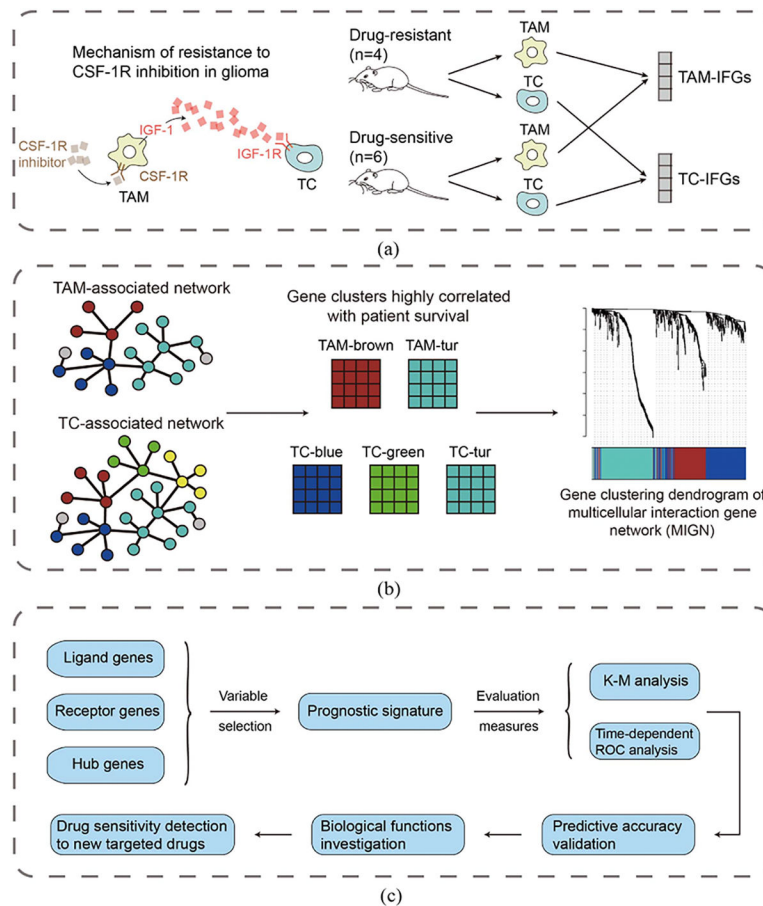
The gene expression datasets as well as clinical information of the patients were downloaded from publicly available databases including the TCGA database, the CGGA database or the GEO database, as described in the main text. The code for MIGN is available at <https://github.com/Xinwei313/MIGN.git>.

References

- [1]. Louis DN et al. , "The 2007 WHO classification of tumours of the central nervous system," *Acta Neuropathol*, vol. 114, no. 2, pp. 97–109, Aug. 2007. [PubMed: 17618441]
- [2]. Jakola AS et al. , "Surgical resection versus watchful waiting in low-grade gliomas," *Ann. Oncol*, vol. 28, no. 8, pp. 1942–1948, Aug. 2017. [PubMed: 28475680]
- [3]. Adamson C et al. , "Glioblastoma multiforme: A review of where we have been and where we are going," *Expert Opin. Investigational Drugs*, vol. 18, no. 8, pp. 1061–1083, Aug. 2009.
- [4]. Van Meir EG et al. , "Exciting new advances in neuro-oncology: The avenue to a cure for malignant glioma," *CA Cancer J. Clinicians*, vol. 60, no. 3, pp. 166–193, May 2010.
- [5]. Lombardi G et al. , "Clinical management of diffuse low-grade gliomas," *Cancers (Basel)*, vol. 12, no. 10, Oct. 2020, Art. no. 3008. [PubMed: 33081358]
- [6]. Mantovani A, Allavena P, Marchesi F, and Garlanda C, "Macrophages as tools and targets in cancer therapy," *Nature Rev. Drug Discov*, vol. 21, no. 11, pp. 799–820, Nov. 2022. [PubMed: 35974096]
- [7]. Cassetta L and Pollard JW, "Targeting macrophages: Therapeutic approaches in cancer," *Nature Rev. Drug Discov*, vol. 17, no. 12, pp. 887–904, Dec. 2018. [PubMed: 30361552]
- [8]. Ries CH et al. , "Targeting tumor-associated macrophages with anti-CSF-1R antibody reveals a strategy for cancer therapy," *Cancer Cell*, vol. 25, no. 6, pp. 846–859, Jun. 2014. [PubMed: 24898549]
- [9]. Tap WD et al. , "Structure-guided blockade of CSF1R kinase in tenosynovial giant-cell tumor," *New England J. Med*, vol. 373, no. 5, pp. 428–437, Jul. 2015. [PubMed: 26222558]
- [10]. Pyonteck SM et al. , "CSF-1R inhibition alters macrophage polarization and blocks glioma progression," *Nature Med*, vol. 19, no. 10, pp. 1264–1272, Oct. 2013. [PubMed: 24056773]
- [11]. Quail DF et al. , "The tumor microenvironment underlies acquired resistance to CSF-1R inhibition in gliomas," *Science*, vol. 352, no. 6288, May 2016, Art. no. aad3018. [PubMed: 27199435]
- [12]. Butowski N et al. , "Orally administered colony stimulating factor 1 receptor inhibitor PLX3397 in recurrent glioblastoma: An Ivy Foundation Early Phase Clinical Trials Consortium phase II study," *Neuro-Oncol*, vol. 18, no. 4, pp. 557–564, Apr. 2016. [PubMed: 26449250]
- [13]. Gilbert MR et al. , "A randomized trial of bevacizumab for newly diagnosed glioblastoma," *New England J. Med*, vol. 370, no. 8, pp. 699–708, Feb. 2014. [PubMed: 24552317]
- [14]. Ni X et al. , "Interrogating glioma-M2 macrophage interactions identifies Gal-9/Tim-3 as a viable target against PTEN-null glioblastoma," *Sci. Adv*, vol. 8, no. 27, Jul. 2022, Art. no. eabl5165. [PubMed: 35857445]

- [15]. Barabási AL and Albert R, “Emergence of scaling in random networks,” *Science*, vol. 286, no. 5439, pp. 509–512, Oct. 1999. [PubMed: 10521342]
- [16]. Jeong H, Tombor B, Albert R, Oltvai ZN, and Barabási AL, “The large-scale organization of metabolic networks,” *Nature*, vol. 407, no. 6804, pp. 651–654, Oct. 2000. [PubMed: 11034217]
- [17]. Langfelder P and Horvath S, “WGCNA: An R package for weighted correlation network analysis,” *BMC Bioinf.*, vol. 9, no. 559, Dec. 2008, Art. no. 559.
- [18]. Ravasz E, Somera AL, Mongru DA, Oltvai ZN, and Barabási AL, “Hierarchical organization of modularity in metabolic networks,” *Science*, vol. 297, no. 5586, pp. 1551–1555, Aug. 2002. [PubMed: 12202830]
- [19]. Zhang B and Horvath S, “A general framework for weighted gene co-expression network analysis,” *Stat. Appl. Genet. Mol. Biol.*, vol. 4, no. 17, Aug. 2005, Art. no. 17.
- [20]. Langfelder P, Zhang B, and Horvath S, “Defining clusters from a hierarchical cluster tree: The Dynamic Tree Cut package for R,” *Bioinformatics*, vol. 24, no. 5, pp. 719–720, Mar. 2008. [PubMed: 18024473]
- [21]. Zhang Y and Shao Y, “Concordance measure and discriminatory accuracy in transformation cure models,” *Biostatistics*, vol. 19, no. 1, pp. 14–26, Jan. 2018. [PubMed: 28481968]
- [22]. Türei D et al. , “Integrated intraand intercellular signaling knowledge for multicellular omics analysis,” *Mol. Syst. Biol.*, vol. 17, no. 3, Mar. 2021, Art. no. e9923. [PubMed: 33749993]
- [23]. Cheng J, Zhang J, Wu Z, and Sun X, “Inferring microenvironmental regulation of gene expression from single-cell RNA sequencing data using scMLnet with an application to COVID-19,” *Brief Bioinform.*, vol. 22, no. 2, pp. 988–1005, Mar. 2021. [PubMed: 33341869]
- [24]. Maeser D, Gruener RF, and Huang RS, “oncoPredict: An R package for predicting in vivo or cancer patient drug response and biomarkers from cell line screening data,” *Brief Bioinform.*, vol. 22, no. 6, Nov. 2021, Art. no. bbab260. [PubMed: 34260682]
- [25]. Iorio F et al. , “A landscape of pharmacogenomic interactions in cancer,” *Cell*, vol. 166, no. 3, pp. 740–754, Jul. 2016. [PubMed: 27397505]
- [26]. Sun X, Liu X, Xia M, Shao Y, and Zhang XD, “Multicellular gene network analysis identifies a macrophage-related gene signature predictive of therapeutic response and prognosis of gliomas,” *J. Transl. Med.*, vol. 17, no. 1, May 2019, Art. no. 159. [PubMed: 31097021]
- [27]. Zhang M, Wang X, Chen X, Zhang Q, and Hong J, “Novel immune-related gene signature for risk stratification and prognosis of survival in lower-grade glioma,” *Front. Genet.*, vol. 11, no. 363, Apr. 2020, Art. no. 363. [PubMed: 32351547]
- [28]. Tirrò E et al. , “Prognostic and therapeutic roles of the insulin growth factor system in glioblastoma,” *Front. Oncol.*, vol. 10, Feb. 2021, Art. no. 612385. [PubMed: 33604294]
- [29]. Abdelfattah N et al. , “Single-cell analysis of human glioma and immune cells identifies S100A4 as an immunotherapy target,” *Nature Commun.*, vol. 13, no. 767, Feb. 2022, Art. no. 767. [PubMed: 35140215]
- [30]. Liberzon A et al. , “The molecular signatures database (MSigDB) hallmark gene set collection,” *Cell Syst.*, vol. 1, no. 6, pp. 417–425, Dec. 2015. [PubMed: 26771021]
- [31]. Verhaak RG et al. , “Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1,” *Cancer Cell*, vol. 17, no. 1, pp. 98–110, Jan. 2010. [PubMed: 20129251]
- [32]. Lu Y and Shao Y, “Multicellular biomarkers of drug resistance as promising targets for glioma precision medicine and predictors of patient survival,” *Cancer Drug Resistance*, vol. 5, no. 2, pp. 511–533, Jun. 2022. [PubMed: 35800363]
- [33]. Cooper LA et al. , “The proneural molecular signature is enriched in oligodendrogliomas and predicts improved survival among diffuse gliomas,” *PLoS One*, vol. 5, no. 9, Sep. 2010, Art. no. e12548. [PubMed: 20838435]
- [34]. Depner C et al. , “EphrinB2 repression through ZEB2 mediates tumour invasion and anti-angiogenic resistance,” *Nature Commun.*, vol. 7, Jul. 2016, Art. no. 12329. [PubMed: 27470974]
- [35]. Wu S, Miao K, Wang L, Ma Y, and Wu X, “Bioinformatics analysis of C3 in brain low-grade gliomas as potential therapeutic target and promoting immune cell infiltration,” *Med. Oncol.*, vol. 39, no. 2, Jan. 2022, Art. no. 27. [PubMed: 35018510]

- [36]. Guo G et al. , “A TNF-JNK-Axl-ERK signaling axis mediates primary resistance to EGFR inhibition in glioblastoma,” *Nature Neurosci.*, vol. 20, no. 8, pp. 1074–1084, Aug. 2017. [PubMed: 28604685]
- [37]. Li P, Xu G, Li G, and Wu M, “Function and mechanism of tumor suppressor gene LRRC4/NGL-2,” *Mol. Cancer*, vol. 13, Dec. 2014, Art. no. 266. [PubMed: 25526788]
- [38]. Feng J et al. , “Leucine-rich repeat containing 4 act as an autophagy inhibitor that restores sensitivity of glioblastoma to temozolomide,” *Oncogene*, vol. 39, no. 23, pp. 4551–4566, Jun. 2020. [PubMed: 32372061]
- [39]. Zhou K et al. , “FXRD mRNA expression represents a new independent factor that affects survival of glioma patients and predicts chemosensitivity of patients to temozolomide,” *BMC Neurol.*, vol. 21, no. 1, Nov. 2021, Art. no. 438. [PubMed: 34753441]
- [40]. He R et al. , “circGFRA1 and GFRA1 act as ceRNAs in triple negative breast cancer by regulating miR-34a,” *J. Exp. Clin. Cancer Res*, vol. 36, no. 1, Oct. 2017, Art. no. 145. [PubMed: 29037220]
- [41]. Wang QW, Lin WW, and Zhu YJ, “Comprehensive analysis of a TNF family based-signature in diffuse gliomas with regard to prognosis and immune significance,” *Cell Commun Signal*, vol. 20, no. 1, Jan. 2022, Art. no. 6. [PubMed: 35000592]
- [42]. Xu Z, Zhang Q, Luh F, Jin B, and Liu X, “Overexpression of the ASPM gene is associated with aggressiveness and poor outcome in bladder cancer,” *Oncol. Lett*, vol. 17, no. 2, pp. 1865–1876, Feb. 2019. [PubMed: 30675249]
- [43]. Grimm D et al. , “The role of SOX family members in solid tumours and metastasis,” *Semin Cancer Biol*, vol. 67, no. Pt 1, pp. 122–153, Dec. 2020. [PubMed: 30914279]
- [44]. Caja L et al. , “Snail regulates BMP and TGF β pathways to control the differentiation status of glioma-initiating cells,” *Oncogene*, vol. 37, no. 19, pp. 2515–2531, May 2018. [PubMed: 29449696]
- [45]. Buschdorf JP et al. , “Brain-specific BNIP-2-homology protein Caytaxin relocates glutaminase to neurite terminals and reduces glutamate levels,” *J. Cell Sci*, vol. 119, no. Pt 16, pp. 3337–3350, Aug. 2006. [PubMed: 16899818]
- [46]. Gu X et al. , “Efficient inhibition of human glioma development by RNA interference-mediated silencing of PAK5,” *Int. J. Biol. Sci*, vol. 11, no. 2, pp. 230–237, Jan. 2015. [PubMed: 25632266]
- [47]. Thompson EM and Stoker AW, “A review of DUSP26: Structure, regulation and relevance in human disease,” *Int. J. Mol. Sci*, vol. 22, no. 2, Jan. 2021, Art. no. 776. [PubMed: 33466673]

**Fig. 1.**

Schematic illustration of the translational pipeline to construct the multicellular interaction gene network (MIGN) for identification of prognostic signature. (a) Detection of immunotherapy-related feature genes (IFGs) for TAMs and TCs from preclinical mouse trials. (b) After translating IFGs to the human genome, TAM- and TC- associated networks were constructed, survival-correlated gene clusters were selected, and the MIGN was built to model the TAM-TC interplay. (c) Based on the MIGN, we identified prognostic signature that comprised of ligands, receptors, and hub genes. Kaplan-Meier and time-dependent ROC analyses were used to evaluate its predictive accuracy. An independent LGG cohort was used for validation. Literature survey was conducted to explore biological functions of the signature genes. Potential drug sensitivity to new targeted therapies was further assessed based on the identified signature.

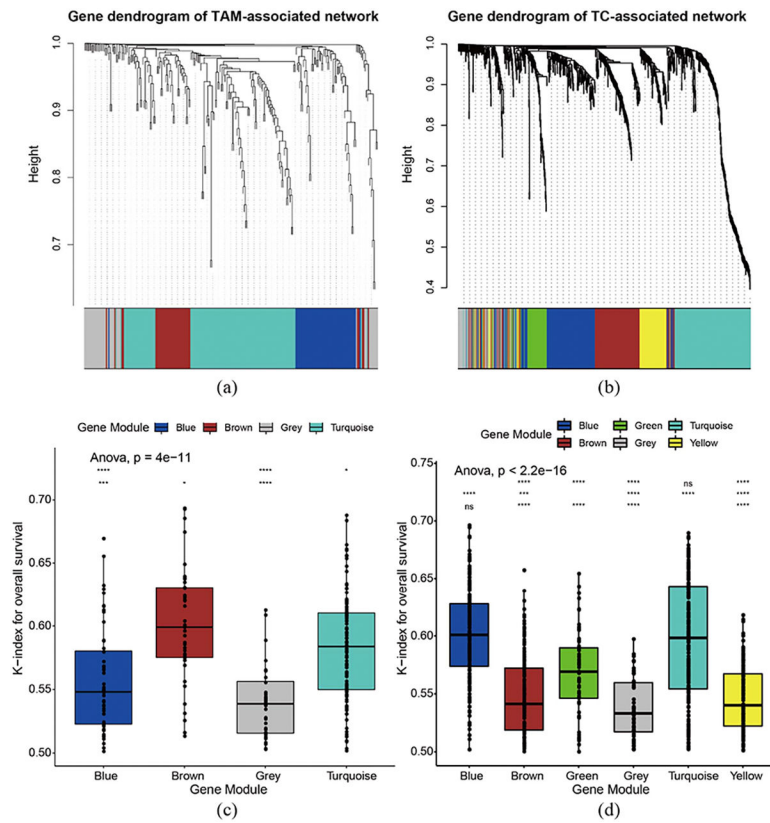
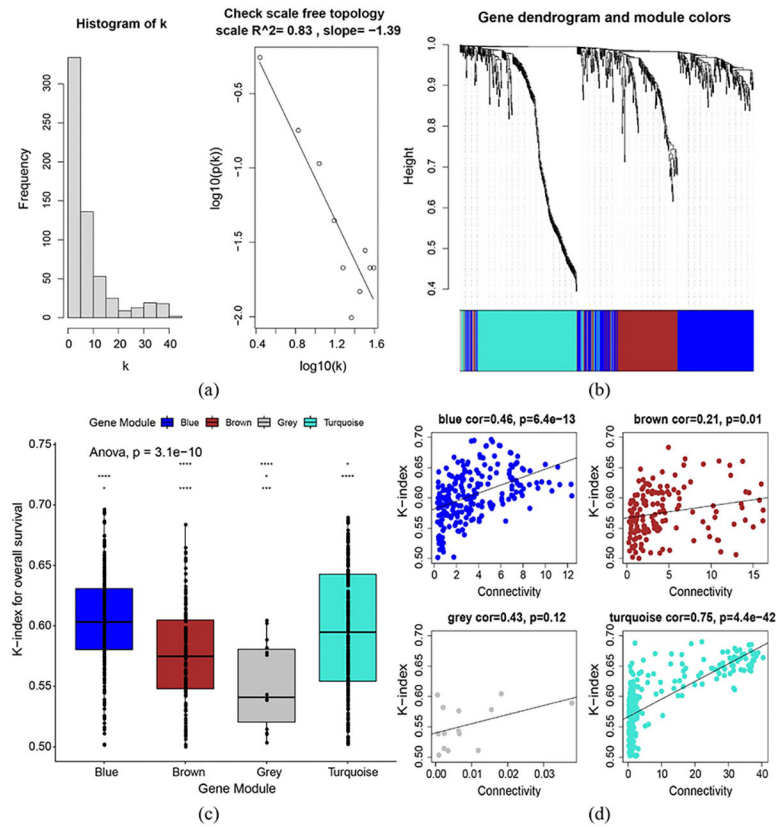


Fig. 2. Clustering analysis of TAM- and TC- associated networks. (a)-(b) Gene clustering dendrogram together with assigned module colors of TAM- and TC- associated networks. (c)-(d) Boxplots representing the distribution of K-index for genes in each module of the corresponding networks.

**Fig. 3.**

Construction and analysis of the multicellular interaction gene network (MIGN). (a) The left panel shows a histogram of network connectivity, and the right panel shows its log-log plot. (b) Gene clustering dendrogram together with assigned module colors. There are 4 clusters in the MIGN, including MIGN-blue, MIGN-brown, MIGN-turquoise and MIGN-grey. (c) Boxplots representing the distribution of K-index for genes in each module. (d) Scatterplot of K-index (y-axis) vs connectivity within the module (x-axis).

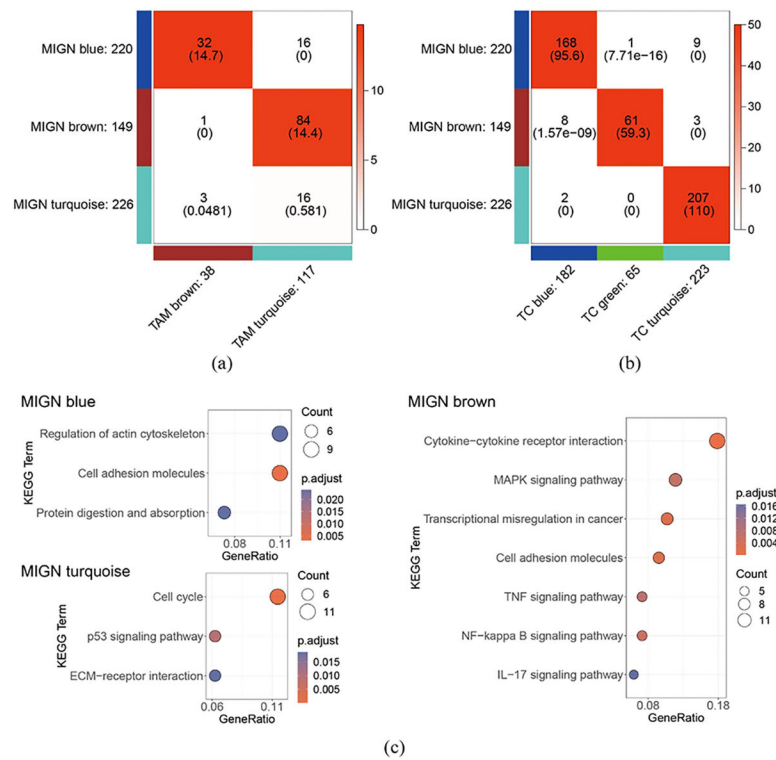


Fig. 4. Cluster correspondence and enrichment analysis. (a) Correspondence of gene clusters between MIGN and the TAM-associated network. (b) Correspondence of gene clusters between MIGN and the TC-associated network. The MIGN clusters with their sizes were spread as columns. TAM and TC clusters with their sizes were spread as rows. The number of overlapped genes for a given pair of MIGN and TAM/TC clusters was calculated, and the statistical significance of the overlap was examined by Fisher's exact test, for which p-value was shown in the parenthesis (in $-\log_{10}$ scale). The color scale represents the p-value of Fisher's exact test: the darker the color, the lower the p-value and the stronger significance of the overlap. (c) KEGG enrichment analysis of gene clusters in MIGN.



Fig. 5. Forest plot of multivariate Cox regression analysis indicated that those ligands (EFNB2, C3, IGF1, TNF), and clinical factors (Age and Grade) were significant by setting the cutoff of p-value as 0.15 in the stepwise variable selection procedure.

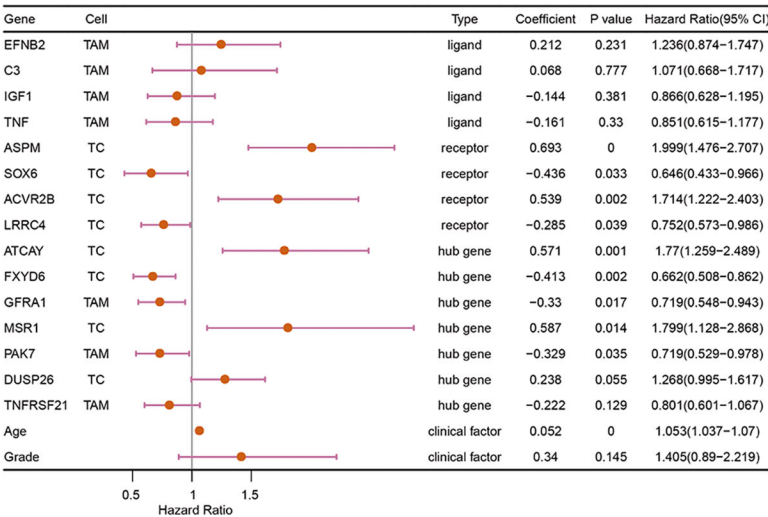


Fig. 6. A 15-gene signature adjusted with clinical factors (Age and Grade) identified in this study for prognosis prediction of LGG patients. The MIGN signature was comprised of 3 types of genes (ligand genes, receptor genes, and hub genes) from either TAM or TC. The forest plot together with coefficient, p-value, and hazard ratio in multivariate Cox regression model were presented.

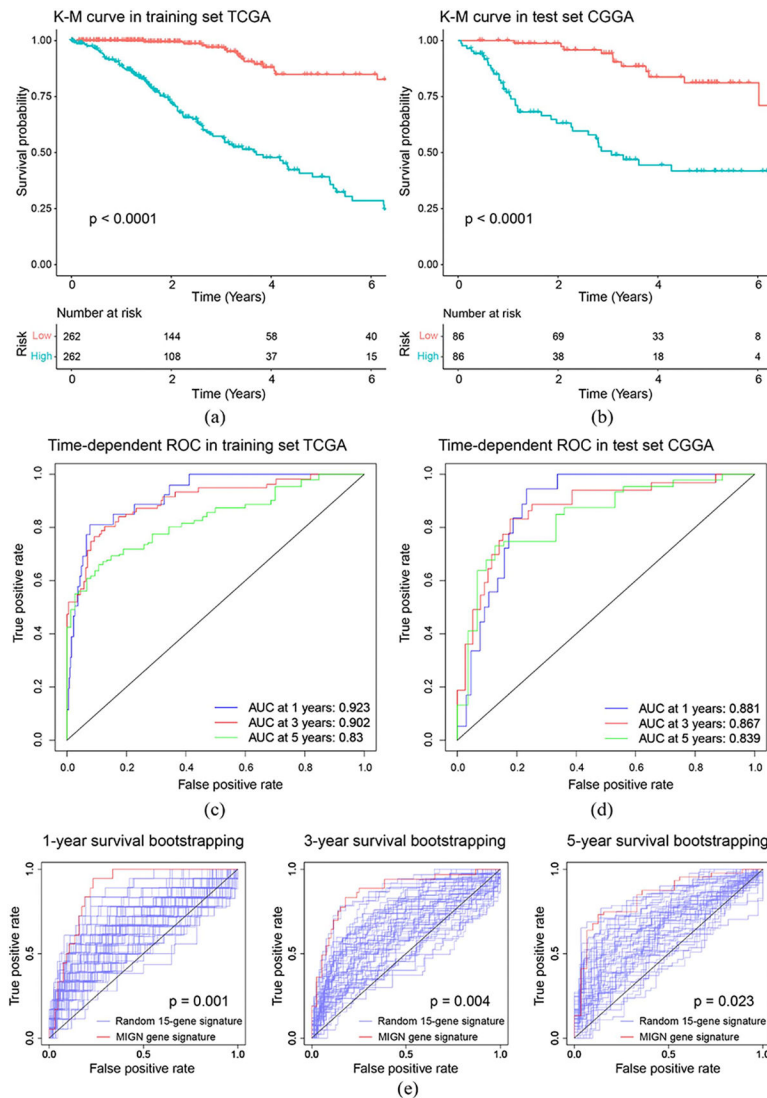
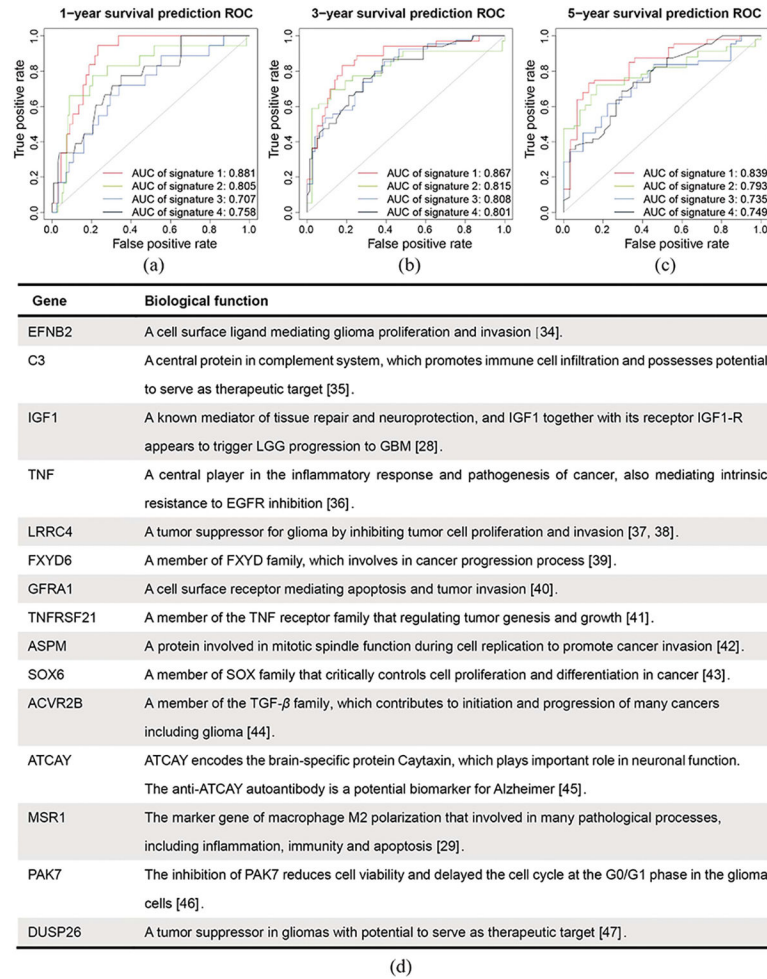


Fig. 7. Evaluation of prognostic performance of the MIGN signature. (a)-(b) K-M survival analysis of high and low-risk groups of LGG patients in the training dataset TCGA and in the validation dataset CGGA. (c)-(d) Time-dependent ROC analysis of LGG patients in the training dataset TCGA and in the validation dataset CGGA. (e) ROC curves of bootstrapped 15-gene signatures (blue, only 100 curves were shown) and MIGN signature (red) with respect to 1-year, 3-year, and 5-year survival rates in the validation dataset CGGA.

**Fig. 8.**

Comparison and functional investigation of the MIGN signature. (a)-(c) The time-dependent ROC with respect to 1-year, 3-year, and 5-year survival rates in the validation dataset CGGA. Signature 1 denotes the MIGN signature proposed in this study. Signature 2 represents the macrophage-related gene signature proposed by Sun et al [26]. Signature 3 is the immune-related gene signature proposed by Zhang et al [27]. Signature 4 denotes the clinical factor signature, i.e., age and grade. The AUC values of each signature are shown at right bottom. (d) Literature survey for the genes involved in the MIGN signature.

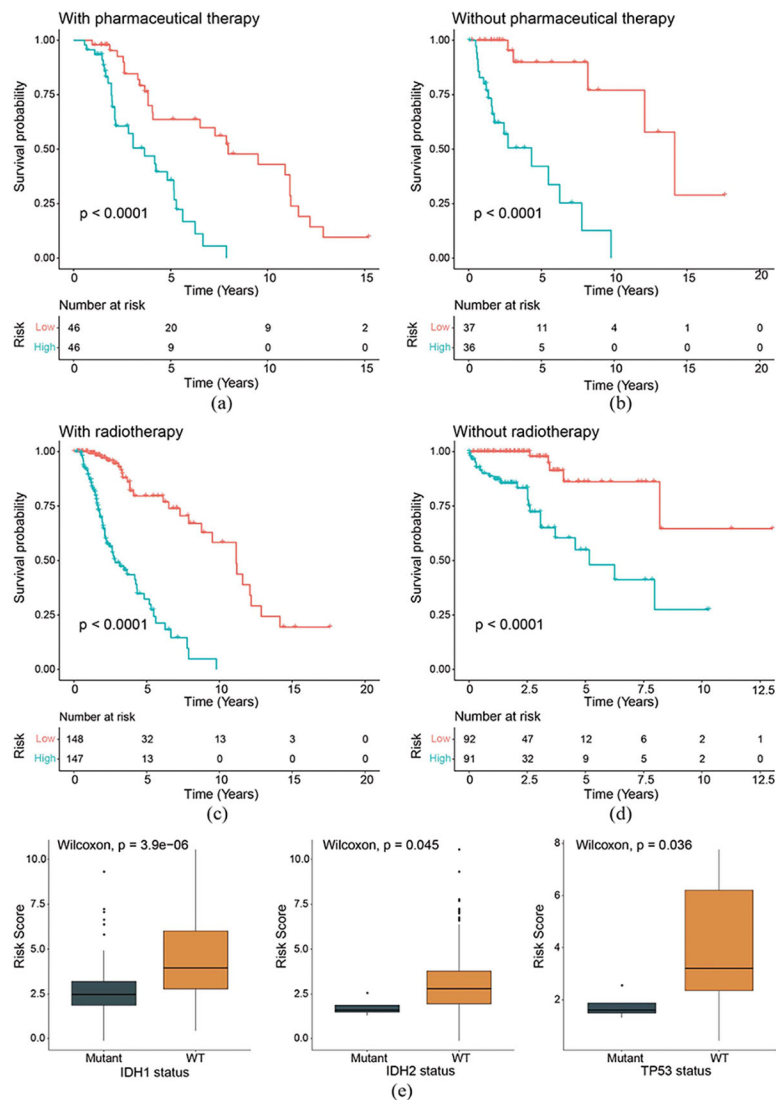
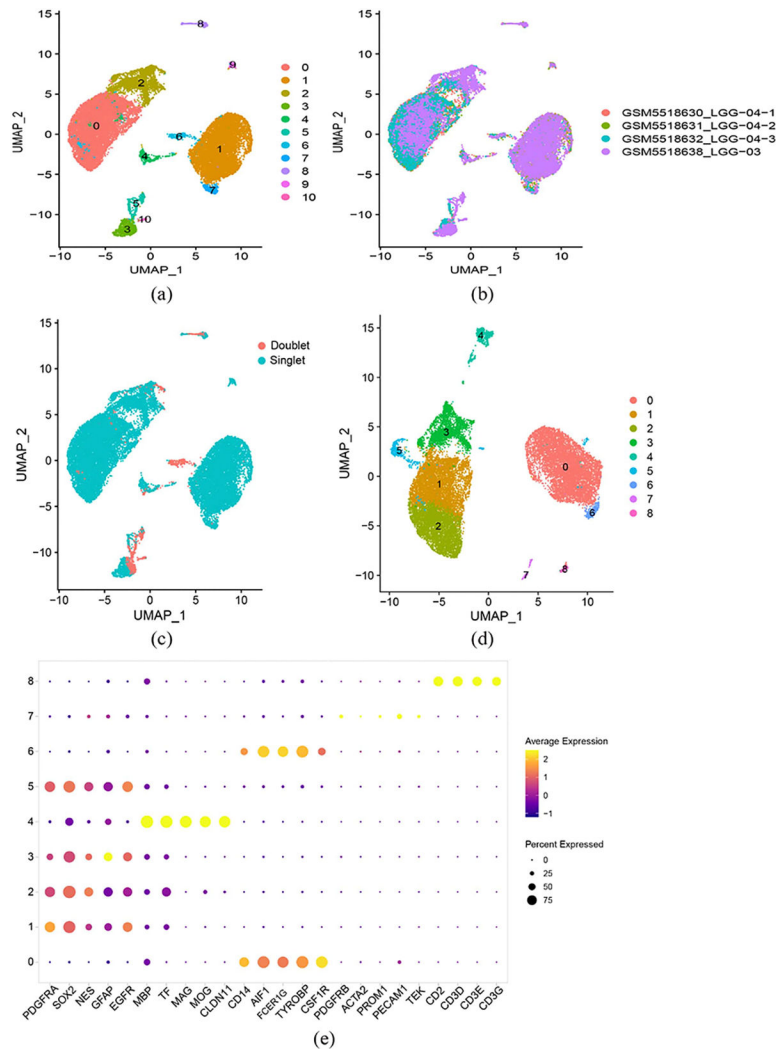


Fig. 9. Prognostic significance of the MIGN signature in the stratified cohorts. The MIGN gene signature retained prognostic significance for LGG patients treated with or without pharmaceutical therapy (a) and (b) and radiotherapy (c) and (d). Median cutoff values were used to determine high-risk and low-risk groups in each stratified cohort. The statistical significance of the difference between survival curves was assessed using the log-rank test. (e) Distribution of the MIGN signature in LGG patients stratified by IDH1 mutation status, IDH2 mutation status, and TP53 mutation status. Wilcoxon rank-sum test was used to assess the statistical significance of the difference between the mutant and wild-type groups.

**Fig. 10.**

Cell type annotation on scRNA-seq data. (a) PCA was applied to reduce the dimensionality of the data for cell clustering. (b) Computed principal components were batch-corrected for variations between tumor specimens. (c) Identification of potential single-cell doublets. (d) Cell clustering map after removing doublet cells. (e) Dot plot showing marker gene expression for different cell types. Dot sizes indicate the percentage of cells in each cluster expressing the gene, and colors indicate average expression levels.

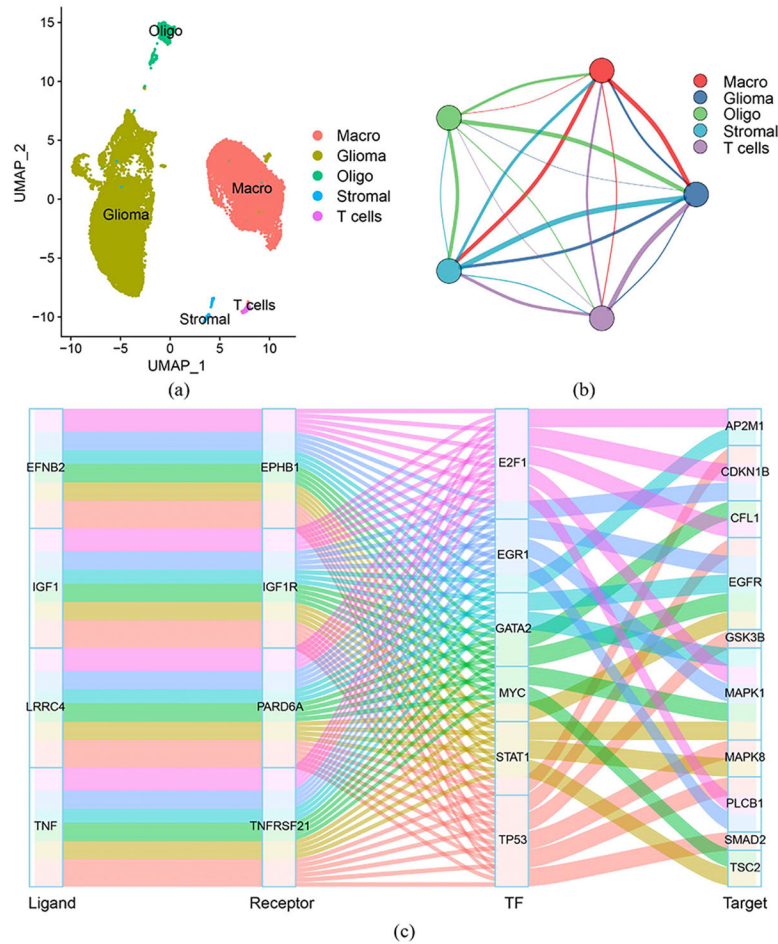
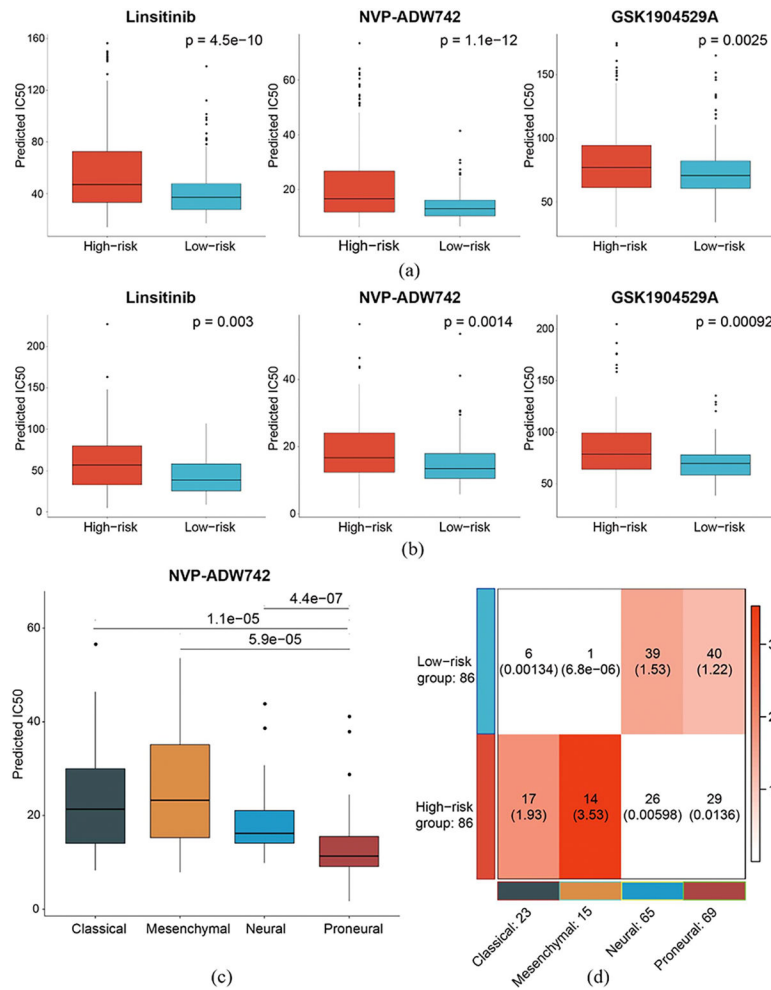


Fig. 11. Verification of interactions between macrophages and gliomas using scRNA-seq data. (a) UMAP projections of 25986 single cells from human LGGs, with cell type annotations. (b) Cell-cell communication networks in glioma microenvironment ecosystem. Each cell type corresponds to a dot of one color, and the communication between two types of cells is represented by the edges, whose thickness represents the number of downstream target genes activated by the ligand-receptor pairs between the cell types, and the color of edge represents the direction of cell communication (e.g., the red edge represents the signals delivered by macrophages). (c) Sankey diagram for the multilayer signaling network. Each rectangle represents a gene, and the connection between genes of different layer is visualized based on the colors that assigned to each TF.

**Fig. 12.**

Assessing drug sensitivity of LGG patients to IGF-1R inhibition stratified by the MIGN signature. (a) Predicted IC50 distribution of three IGF-1R inhibitors for high-risk and low-risk LGG patients in the TCGA dataset. IC50 indicates the half maximal inhibitory concentration. (b) Predicted IC50 distribution of three IGF-1R inhibitors for high-risk and low-risk LGG patients in the CGGA dataset. (c) Predicted IC50 distribution of the IGF-1R inhibitor NVP-ADW742 for four subtypes of LGG patients in the CGGA dataset. (d) Correspondence of samples between LGG subtypes and the risk groups classified by the MIGN signature in the CGGA dataset. The number of overlapped samples for a given pair of risk group and subtype was calculated, and the statistical significance of the overlap was examined by Fisher's exact test, for which p-value was shown in the parenthesis (in $-\log_{10}$ scale).