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Cell type-specific Study to Unveil the Relation between DLBCL and GBM through Blood-Brain-Barrier Disruption

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ABSTRACT

Diffuse large B cell lymphoma is an aggressive and fast-growing form of non-Hodgkin's lymphoma. DLBCL becomes fatal if it is not treated in the early stage. Despite its aggressive nature, 50-70% patients respond successfully, whereas 30-40% patients have an inadequate response to the currently available therapies. DLBCL spread to the brain through spinal fluid. It is becoming a rapid emergency to detect the cell-specific changes of DLBCL metastasis that leads to central nervous system (CNS) disorder. As per our knowledge, the cell-specific studies of genes of DLBCL involved in CNS disorder are still limited. To bridge this gap, we proposed a novel analysis. Firstly, we have identified DLBCL cell-specific gene clusters and considered genes along with their associated pathways that have an impact on blood-brain-barrier disruption. In the second stage, we tried to establish the relation between Diffuse large B cell lymphoma and glioblastoma based on cell-specific pathways. Interestingly it has been observed that high semantic-valued pathways are the prime regulators of GBM. Finally, this study reveals how cell types are responsible for the pathway shifts and also provides a cell-specific channel of triggering the progression of GBM among DLBCL patients.

Introduction

Diffuse large B-cell lymphoma (DLBCL)¹ is the most common lymphoma and nearly 30-40% of lymphoma patients suffer from this disease. DLBCL is initiated from white blood cells. DLBCL patients are found with multiple lumps in the affected areas due to their fast-growing nature. Previous studies reported that the chance of DLBCL has been increased among those who suffer from autoimmune diseases or those who have a weak immune system for some earlier disease. Patients with prior history of radiation and chemotherapy also possess high risks. For a long time, it is considered as a single entity disease. The advancement of different sophisticated techniques, gradually revealed the genetically heterogeneous characteristics of DLBCL². Mainly, there are two groups of DLBCL v.z. i) activated B-cell subtype and ii) germinal centre B-cell subtype³. These two subtypes are related to various B-cell differential stages and encompass distinct oncogenic mechanisms. Although DLBCL is aggressive in nature but it is recoverable with radiation and chemotherapy. The most commonly found treatment strategy is R-CHOP. Despite of well-known available treatment strategies⁴, 50% patients are noticed with inadequate responses to the prognosis⁵. In⁶, the authors show risk assessment and prophylaxis for central nervous system relapse in diffuse large B-cell lymphoma. On the other hand, in a study they found elderly patient diagnosed with DLBCL possess Alzheimer's as a comorbidity disease⁷. It is also noticed that patients those are failed to be diagnosed at an early stage result in long term disorders such as multiple sclerosis cite. Therefore, it is important to understand the cell-cell heterogeneity of DLBCL.

In this regard, the first fold of this study is performed to identify the cell-specific influential markers of DLBCL that able to cross the blood-brain-barrier (BBB). Consequently, any abnormal leak may help to transmit the cell type-specific markers of DLBCL to spread in the CNS and help to form different neuro-diseases. However, it is observed that these cell types along with the markers are found to cross the blood-brain barrier also. On the second fold of the study, we tried to find a relation between two aggressive malignancies. Various studies show that DLBCL patients have high chance of CNS disorder. However, a very crucial malignancy of brain is glioblastoma. From the literature, we found that very few studies are performed to understand this connection between these two aggressive cancers. Due to the lack of cell-specific molecular study, the knowledge regarding the path through which DLBCL helps in GBM progression is still unclear. To bridge this knowledge gap, we proposed a framework in this article. To provide an insight into this connection we conducted a single-cell level study of glioblastoma also. From the previous fold we identified DLBCL cell types those are involved in CNS disorder. Those cell types are further analyzed to identify the crucial signaling pathway and a pathway semantic network is established to identify the most important pathways and how they connected with each other. Interestingly we found some of the crucial pathways of DLBCL are the key regulator of GBM also⁸. We observed those pathways in details. The cell-specific markers present in both the diseases are considered to perform gene-gene interaction. The interaction network depicts and clearly show that both the disease-specific genes present in the common pathways are highly connected and communicate with each other. From this study we determined the communicating pathways of the two aggressive malignancies as well as the biomarkers. The biomarker of the selected

pathways opens a new direction of research on DLBCL and GBM. Moreover, a detailed understanding of the connection between DLBCL and GBM may provide additional biological relevance in the context of therapeutic studies of these two diseases.

Results

The ScRNASeq data helps to reveal the information regarding DLBCL disease. Seurat clustering algorithm is performed on the curated dataset to identify the cell types. The diverse cell types recognize the functionality of a particular tissue or organ and unzip the heterogeneity of organism into a specific taxonomy. During this study, clusters resulted from three DLBCL patients dataset are twelve, eleven and nine respectively (shown in Figure 1 - 3). Different colours are used to distinguish the clusters. The cell type of each cluster is identified through CellMarker⁹ and PanglaoDB databases¹⁰. The expression level of CD40 in DLBCL patients treated with R-CHOP behaves as an independent prognostic predictor. Therefore we performed a violin plot to understand the expression level of this influential marker in each cell type.

Figure 1. (a) The ScRNASeq data analysis of the first DLBCL patients categorized the cell types into 12 clusters. The clusters are labelled with the name of the cell types. The expression level of CD40 in DLBCL patients treated with R-CHOP behaves as an independent prognostic predictor. Therefore (b) The violin plot is used to show the expression level distribution of CD40 in all the cell types. B-cell and Endothelial cells show high expression of CD40 in first DLBCL patient.

The most important cell types of DLBCL patients are identified by performing a venn diagram of the three patients. As a result, B-cell, T-cell, NK-cell, endothelial and macrophage cell types are found common. We considered these cell type for further pathogenic analysis of DLBCL. All the influential markers of the selected cell types are observed in details in order to identify the cell type-specific pathways. All possible pathways from three patient datasets are considered and pondered from Reactome database¹¹ and KEGG Pathway database¹². Among a huge number of pathways we consider those having maximum number of influential markers and shows crucial impact on DLBCL. During this study, we noticed that most of the pathways are common among the cell types but some are unique and cell type-specific. However, the marker association of the common pathways are cell type dependent. The biological process and molecular function are observed for each associated pathways. Depending on the processes, cell type-specific semantic network is established. The common cell type-specific semantic networks are shown in Figure 4 (the pathway semantic network of NK-cell is shown in supplementary Figure F1). Each coloured node represents a particular pathway and the connecting edges between two nodes depict the similarity value. The cell-specific semantic network helps to reveal the pathways those are triggered by the potential markers and finally leads to metastasis. Furthermore, from literature it is observed that patients suffering from DLBCL are at high risk of brain diseases¹³. For brain diseases, the pathways need to cross the blood brain barrier. In this regard, we studied the pathways in details and noticed that some of the pathways are actively played an crucial role in this process. From the previous knowledge, it is evidenced that immune cells such as B-cell, T-cell, NK- cell, endothelial cell and macrophage have an impact on the progression

of multiple sclerosis, Parkinson's and Alzheimer's diseases¹⁴⁻¹⁷. From the semantic similarity diagram shown in Figure 4, we can depict that important cell-specific pathways of DLBCL also have an impact on the CNS diseases.

Figure 2. (a) The cell types of second DLBCL patient are divided into 11 cluster by ScRNASeq data analysis. (b) Through a violon plot the expression level distribution of CD40 in all the cell types.

Figure 3. (a) The ScRNASeq data analysis of the third DLBCL patients categorized the cell types into 9 clusters. The clusters are labelled with the name of the cell types. (b) The violon plot is used to show the expression level distribution of CD40 in all the cell types.

Consequently, the other objective of this study is to understand if any relation exists between two aggressive malignancies such as DLBCL and glioblastoma. In this regard, we performed a single cell clustering on patient suffered from GBM. As

a result 17 clusters are formed which are labelled with the name of cell types, shown in Figure 5(a). We also identified the cell-specific pathways of the glioblastoma reported in supplementary Table T1. Interestingly, we noticed that most of the pathways reported in Table 1 are the key regulator of glioblastoma progression. Therefore, the detail study of these pathways can reveal the relation between the diseases.

Figure 4. A cell-type-specific pathway semantic similarity networks of (a) B-cell, (b) T cell, (c) Endothelial cell and (d) Macrophages are established by considering the biological process associated with each pathway. The nodes represent a particular pathway and their connecting edges define the weight between two pathways in order to understand the highest sharing biological process. The red marked bold edge in the networks indicate the higher association score between those pathways.

Furthermore, based on the selected pathways, the markers which interact with each other during the DLBCL metastasis and helped in GBM progression are identified along with their cell types. The expression level of the markers from each cell types are shown in Figure 5. In Table 1 we reported all the markers and their respected cell types of two diseases. Moreover, the cell types those are having an interaction with the markers of DLBCL are identified and to showcase the expression level of the markers in the particular cell type we used a violin plot to visualised the expression level of the markers in the selected cell types. The reported markers of GBM shows high expression level on the cell types those are actively involved in the GBM progression and also in the structure formation of BBB.

In order to understand the relation between these two diseases through pathway information we performed an gene-gene interaction network among the markers present on the common pathways of both the diseases shown in Figure 6. During the construction of the network from STRING database, only protein homology, co-expression and experimentally determined based relations are considered. These relations among the markers of two diseases help to understand the route of DLBCL metastasis and how it cross the blood brain barrier and finally contributed to GBM progression.

Discussion

The objective of this study is to identify the cell-specific markers those involve in the disruption of the BBB. Furthermore, recognised the associated pathways of the influential biomarkers that helps to communicate with the GBM-specific cell-types. In this regard, common cell types are identified from three DLBCL patients. Though DLBCL patients become complicated during metastasis and leads to CNS disorder¹⁸, the study focuses on the pathway information considering cell specificity. Secondly, from the cell-specific pathways we identified some signaling pathways those evidenced as a key regulator of GBM progression. From our study we tried to establish the gap to knowledge about the the contribution of DLBCL markers towards the GBM progression.

Figure 5. (a) Glioblastoma single-cell RNAseq data analysis categorized the cell types into 16 clusters. Some important cell types are identified as a targeter during DLBCL metastasis. Through a violin plot the expression level distribution of the markers are showed along with their corresponding cell types. The cell types neural stem cell and natural killer cells are denoted as NSC and NK cells respectively.

Pathway	DLBCL	GBM
MAPK Signaling	B-cell (STMN1), Endothelial (FLT3)	Pericytes (PDGFRB), Endothelial (JUND), Glial (FGFR2), Reg T-cell (IL1B, EREG), Astrocytes (FLNA, VEGFA), M2 macrophage (CSFIR, DUSP2, IL1B, NFKB1, IL1B, CD14), Fibroblast (TGFB2)
PI3K-Akt signaling	Endothelial (FLT3), B-cell (CDK4)	Pericytes (COL1A1, LAMC3, ITGA8, COL6A3), T-cell (IL2RG, IL7R), Endothelial (YWHAQ), Glial (FGFR2), Reg T-cell (EREG), Astrocytes (TNC, E1F4EBP1, FN1, VEGFA, HSP90AA1, SGK1, MCL1), M2 macrophage (CSFIR, NFKB1)
NFkB-signaling	Endothelial (CD40, CCL19)	M2 macrophage (CXCL8, IL1B, CD14, NFKB1), Astrocyte (VCAM1, NFKB1, GADD45B), Reg T-cell (CXCL8, BCL2A1, IL1B), T-cell (LTB)
RAS signaling	Endothelial (FLT3)	Pericytes (PDGFRB), M2 macrophage (CSFIR, REL, ABL2, NFKB1) Astrocytes (PLA2G5, VEGFA), Reg T-cell (CALM2, ETS2), Glial (RAPGEF5, FGFR2)
P53 signaling	B-cell (CCNB2, CCNB1), T-cell (CDK4, CDK1, GTSE1)	Neural stem cell (RPM2), Astrocytes (IGFBP3, GADD45B), M2 macrophage (CDK1)
Hippo signaling	Endothelial (ACTB, RASSF4), B-cell (BIRC5)	Fibroblast (SIX2, TGFB2), Endothelial (YWHAQ, ACTG1)
JAK-STAT signaling	Endothelial (CSF2RA)	Pericytes (PDGFRB), T-cell (STAT4, IL2RG, IL7R), Astrocytes (SOCS3, MCL1), Fibroblast (FHL1, GFAP)
T-cell receptor signaling	Memory T-cell (PTMA, CD8B, CD8A)	M2 macrophage (LCP2, NFKB1), Astrocytes (NFKB1A, JUN, FOS) T-cell (FTPRC, CD247, CD3E, CD3D)

Table 1. The genes along with their respective cell types present in the same signaling pathway of DLBCL and GBM disease.

Role of cell type-specific pathways in DLBCL in disruption of Blood Brain Barrier

In our study we found some cell types such as B-cell, T-cell, NK-cell, endothelial cell and macrophage. B-cell possess multiple functionality and also transform into various pathways. Due to the heterogeneous functionality, B-cell represent a diverse category of diseases those are varying pathologically as well as clinically. The malignant growth of this cell-type at various stages are responsible for B-cell lymphoma. Similar like other lymphomas this type is also resulted due to the genetic mutations of both the normal genes or proto-oncogenes and tumor suppressor genes.

During the study, we observed some important markers such as LEF1, TCF7, CD79A and CD79B. The first two are from T cell whereas other two are from B-cell respectively. Each cell type possesses significant pathways with their unique implications on the progression of DLBCL (e.g., Wnt signalling pathway, NOD-like receptor signaling pathway, Prolactin signaling pathway and Chemokine signaling pathway etc.). The first two aforementioned markers have worked as a downstream transcription factor for WNT signaling pathway¹⁴. This pathway plays a key role in the development and maintenance of embryo, metabolism and cellular growth¹³ as well as involves in the progression of carcinoma¹⁸. During the development of T cells from T cells, the expressions of these two markers are decreased. Our detailed study of this cell type reported another pathway i.e cytokine-cytokine interaction. As the selected cells are part of initial immune response, the cells have been activated after detection of the antigen. Initially, the malignant and/or infected cells are fatally targeted by secreting cytokines¹⁵. However, disruption during the secretion may lead to pathogenic outcome e.g., multiple sclerosis. Furthermore, Reya et al¹⁶ has reported that deficiency of LEF-1 hampers the proliferation and survival rate of pro-B cells where LEF-1 contributes in the inability of the cells to respond to mitogenic signals of WNT in the micro environment. WNT signaling pathway is directly connected to the BCR signaling pathway which is the key regulator of B cell. Normal BCR signaling is associated with the proliferation, survival, apoptosis, and differentiation of B-cell. However, abnormal activation of this pathway is responsible for oncogenesis, e.g, DLBCL¹⁹. Interestingly, two markers (CD79A, CD79B), reported previously, are the key player of BCR signaling pathway in B-cell. In a subtype of DLBCL, mutation of these two markers are observed which leads to BCR signaling pathway dysregulation and finally leads to oncogenesis. From the B-cell pathway list, we found another marker CD40 which is significantly associated with multiple pathways. The reported marker is a surface receptor that can control the activity of bruton tyrosine kinase (BTK) in B cell¹⁷. The expression of BTK is restricted. However, the pathway is involves in the differentiation and activation of the cell type. Also related to immune function, CD40 is responsible for transcription regulation, and apoptosis modulation in both Toll-like receptor (TLR) pathway and cytokine receptor signaling

inhibits the function of important cell type, such as T cells and B cells through variable mechanism.

Another cell type, natural killer cells are inhibited by regulatory T cell. Both the T cells and NK cells are cytotoxic effector cells of the immune system. However, their specificity, sensitivity, and memory mechanisms are drastically different. A very little information is available of NK cell types. From the literatures^{23,24}, it is observed that marker IRF1 can critically regulate TNF signalling pathway. Interestingly, we also noticed the association between a marker and the pathway in NK cell type. The complex environment of DLBCL possesses TNF- α along with chemokines and some cytokines produced in mesenchymal matrix from different inflammatory cells. In a recent study, it has been proved that sCXCL16 can reinforce TNF- α to mediate the DLBCL cell proliferation²⁵. In this process, NF- κ B signaling is activated by sCXCL16 to increase the production of TNF- α in DLBCL cells.

Along with the discussed pathways above, some other significant pathways are also observed during the detailed study of cell-specific pathways. These pathways are also associated with NF- κ B signaling pathway and dedicatedly possess an important role in DLBCL. Among these, PI3K/AKT/mTOR signaling pathway is found activated in most of the DLBCL patients. The phosphorylation of phosphatidyl-inositol-4,5-bisphosphate converted to phosphatidylinositol-3-4-5 triphosphate (PIP3) by the activated PI3K isoform. This conversion activates AKT which plays an important role in PI3K/AKT/mTOR pathway. Activated AKT (p-AKT) effects the signals of pro-survival for anti-apoptosis, proliferation and cell growth through mTOR³⁰. Study of genetic ablation of BCR signaling in B-cell unveil that survival of mature B-cell with BCR deficiency can be retrieved by downstream PI3K signaling. Similarly, downstream kinase PDK1 as well as activated PI3K are crucial for the survival of cell lines that possess CD79B mutation. Inhibition of this activated pathways can be a promising target for improving DLBCL therapeutic research in future. Also, TLR signaling pathway plays as a stimulus to activate NF- κ B in ABC-DLBCL (a subtype of DLBCL). Toll-like receptor (TLR) signaling is mediated by an adaptor protein MYD88³¹. Another pathway reported under cell type T cell is NOD-like receptor (NLR). This is responsible for multiple biological functions. These functions include initiating the formation of nuclear factor κ B (NF- κ B), stress kinases, interferon reaction factors (IRFs), inflammatory caspases and autophagy. Among the subfamilies of NLRs depending on the nature of N-terminals residues, an acidic transactivation domain (AD) or NLRA subgroup shows significant role in DLBCL.

DLBCL Cell type specific pathways involved in Glioblastoma

From the previous section we noticed that DLBCL patients have a tendency to develop central nervous system (CNS) disorder. However, the pathways which cross the blood-brain-barrier to help in the progression of the diseases are still unknown. Among the aforementioned pathways, alternation in BCR, TCR and NF- κ B pathways are shown in greater than 90% of primary CNS lymphoma³². Due to the metastasis of DLBCL a very rare type of CNS lymphoma is described as a subtype of DLBCL lymphoma. In this microenvironment, the cancer cells are developed from lymph tissue in the brain or spinal cord (primary CNS) area or even metastasis to the brain from other parts of the body (secondary CNS). Another type, leptomeningeal lymphoma is observed in this kind of patients which affect the spinal fluid that bathes the brain and spinal cord. This subtype is mostly considered as a metastasis form of diffuse large B-cell lymphomas and the patients show very poor prognosis³³. Apart from CNS diseases, DLBCL patients may affect by many other brain's related diseases for example Head and Neck carcinoma. In a review paper, the authors reported that NF- κ B, Wnt/ β -catenin and PI3-K/AKT/mTOR signalling pathways are involved in Head and Neck carcinoma³⁴ which also have an important impact on DLBCL. Another very common brain malignancy is glioblastoma. Due to the aggressive nature of this cancer type spreads very quickly and have no cure³⁵. Though DLBCL and GBM is mostly found in adults and have a fast-growing characteristic so we tried to investigate if any relation exists between the two diseases. To uncover the doubts, we performed a comparative study of these two diseases at the single-cell level. The influential markers along with their cell types of DLBCL are further considered. Similarly, the cell type-specific marker genes of GBM are selected and their corresponding pathways are also determined. From the cell type-specific pathway semantic similarity networks DLBCL, few pathways are observed as a key regulator of GBM. In Table. 1, the crucial pathways are reported along with the cell-specific markers of two diseases. For a better understanding of the communication of the two diseases we established a reported pathway-specific gene-gene interaction network. The networks depict that, Among the reported pathways, the impact of NF- κ B signaling pathway on DLBCL patients are already discussed in the previous section.

NF- κ B signaling pathway, one of the important pathways in DLBCL³⁰. NF- κ B transcription factor regulates various aspects of immune functions and plays a pivotal role in inflammatory responses. We found a marker CD40, which belongs to the endothelial cell type actively present in this pathway. Apart from DLBCL, this pathway plays a crucial role in GBM also. Mainly, two major mechanisms are present to activate NF- κ B in glioma. Firstly, NF- κ B is activated through EGFR signaling. The mutation of the EGFR gene is very common in GBM and found in most of the patients³⁶. Secondly, 790 glioblastoma patients showed 23.4% rate of deletion of the NFKB1A gene that is responsible for encoding *I κ B α* , reported in a study. However, *I κ B α* is the member of cellular proteins that control the inhibition of the NF- κ B transcription factor. This helps to keep the NF- κ B inactive in the cytoplasm and blocks them from DNA binding. Therefore, the deletion of the NFKB1A gene activated the consecutive NF- κ B. Moreover, activation of NF- κ B is reported to support a mesenchymal phenotype in

glioblastoma. A significant study suggests that NF- κ B possess an important role in GBM pathogenesis and the NF- κ B signaling pathway contribute in the prognosis also. Interestingly, the classical NF- κ B signaling is the downstream of the T-cell receptor (TCR) signaling pathway and BCR signaling pathway. Consequently, mutation or overexpression of EGFR activated the PI3K signaling as well as MAPK signaling pathway during GBM. The downstream PI3K signaling pathway is associated with NF- κ B signaling pathway and dedicatedly possess an important role in GBM. Another important marker FLT3 is found highly expressed in multiple pathways. Moreover, the pathway-specific gene-gene interaction networks depict that these marker possess a crucial role in establishing a connection between two diseases. FLT3 is also expressed in PI3K signaling pathway. Whereas, MAPK signaling pathway shows high expression rate of two influential markers such as FLT3 and EGFR. MAPK signaling pathway is equally important in both the malignancies due to its role in gene expression regulation, controlling cellular growth and survival of the cells³⁴. Therefore, abnormalities in this pathway may increase cell proliferation and leads to uncontrolled resistance to apoptosis. Moreover, activated epidermal growth factor receptor and MAPK initiates the downstream induction of transcription factors, such as nuclear factor- κ B (NF- κ B). Furthermore, MAPK signaling pathway is actively participated in glial cell formation. This cell type is targeted by DLBCL markers shown in previous section. This information infer the connection between DLBCL and GBM and how they contributed towards each other metastasis. Additionally, FLT3 is found in RAS signaling during this study. The gene-gene interaction unfold that genes from both diseases belong to this pathway. A study shows that this signaling pathway is critically important for maintaining glioblastoma³⁷. This also demonstrates that continual suppression of the pathway is required and necessary to suppress the tumorigenic potential of glial progenitor cells. It is shown in the interaction network that two genes such as RAPGEF5 and FGFR2 belong to glial cell types are interact with endothelial cell-specific gene FLT3. This indicates that the FLT3 gene during DLBCL alter the RAS signaling pathway and increases the tumorigenic potentiality of a glial cell through two previous mentioned genes and finally hamper the glioblastoma maintenance. Beside this, Ras induces the NF- κ B in order to upregulate the gene products which are required to overcome Ras-induced apoptosis and potentiate proliferation.

From the semantic similarity network some unique pathways are observed those play an crucial role in both DLBCL and GBM. Among those pathways, Furthermore, activated p53 also reduced the transcription of Cyclin B and hampers the progression of the cell cycle. Interestingly, we noticed these genes during our study as a DLBCL marker belongs to T-cell and B-cell respectively. This is evidenced that p53 is frequently a wild type in DLBCL and the loss of wt-p53 promotes metastasis also³⁸. On the other hand, an altered p53 signaling pathway is found in GBM. So we can deduced that p53 signaling is one of the reasons for DLBCL metastasis and this also helps the DLBCL markers to interact with the markers of GBM and initiate the GBM progression. Another important pathway is JAK-STAT signaling pathway. From literature, it is evidenced that, this pathway acts as a molecular hub in glioma, astrocytes, stromal and immune cells, under the critical microenvironment of the cellular population³⁹. In many studies, the reported GBM markers are found as a key regulator of this pathway that finally resulted in GBM⁴⁰. In endothelial and B-cell types of DLBCL, the Hippo-YAP signaling pathway is noticed. It is also shown that the expression level and nuclear gathering of Hippo-Yes-associated protein (YAP) in DLBCL can be notably controlled by insulin-like growth factor-1 receptor (IGF-1R) knockdown. However, this signaling pathway is crucial in brain development and contributes to glioblastoma also⁴¹.

Conclusion

In this article, influential cell types for DLBCL are identified based on single-cell bioinformatics strategy. The objective is to identify the potential markers responsible for the pathway shift in the particular cell type. Moreover, these pathways are studied thoroughly to reveal the path through which DLBCL metastasis is progressed and activated diverse CNS diseases, specially GBM. Initially, we have detected the cell types of DLBCL from three patients and performed a venn diagram to identify what are the common cell types and the most commonly found among the DLBCL patients. The markers of each cell type from each patient are considered as an influential marker and their impact on biological pathways are also identified. Additionally, the connection between DLBCL and GBM is established. DLBCL patients may undergo various types of neurodegenerative diseases as well as CNS disorders. However, the patients may also tend GBM progression is not clear yet. During this study, we found common pathways between DLBCL and GBM. Those are mostly interconnected with each other. The two important pathways of DLBCL such as T-cell receptor and B-cell receptor are noticed indirectly controlling all the key pathways of GBM. Therefore, we can conclude that the pathways promote DLBCL metastasis help in GBM progression also. Furthermore, the gene-gene interaction network shown in this article decipher that through the discussed pathways the DLBCL markers can interact with the influential genes of GBM and help during the propagation of the diseases. Therefore, the identified markers and pathways can be used as a therapeutic candidate for both the malignancies.

Methods

Publicly available single-cell RNAseq datasets of three DBLCL and one GBM patients are downloaded from diverse websites. The proposed framework of this study is described in Figure 7.

Figure 7. The flowchart of the proposed framework

Dataset Preparation and Cell types Identification

The raw count matrix is processed by Seurat V3.0⁴². The undesired cells from the downloaded datasets are filtered and cells with unique feature counts over 2,500 or less than 200 are selected. Furthermore, >5% mitochondrial counts are also considered for filtering the cells. After performing the previous step, "LogNormalize" function is used in order to normalize the data. In this method, the data is scaled by multiplying a value 10,000. Some genes are dedicatedly show high expression value in some particular cells. It is also found that those expressed genes are important to understand the biological signal in single-cell dataset. These genes are identified by "FindVariableFeatures" function. As a result 2000 features per dataset are returned which are used in principal component analysis (PCA). The score of PCA are used to construct the K-nearest neighbors (KNN) graph. Based on the graph, cells are iteratively grouped together along with optimizing the standard modularity function, "FindCluster". This clustering function implements Louvain Algorithm and visualised by UMAP techniques. The differentially expressed genes of each resulted cluster are identified by using "FindMarker" function along with predefined default percentage value set to .25. Finally, the cluster-specific genes are considered for further study.

Pathway Semantic similarity calculation

The pathways of the potential markers of each cell types are utilized for computing the pathway semantic network. For the selected cell type-specific pathways a set of biological process are considered. Based on these biological processes the semantic similarity is calculated.

In Wang method, the semantic value is defined as V as the aggregate contribution of all terms in DAG_V to the semantics of term V . To contribute more towards semantic terms need to be closer of V in DAG_V . The GO term V is defined as $DAG_V = (V, S_V, E_V)$, S_T and E_T represent set of GO terms and set of GO terms connecting edges respectively. The term V and its ancestors are included in term S_V . Thus, defined the contribution of a GO term t to the semantic of GO term V as the GO -value of GO term t related to term V . For any of term t in DAG_V , its GO -value related to term V , $GO_V(t)$ is defined

as:

$$\begin{cases} SGO_V(V) = 1 \\ GO_V(t) = \max\{c_e * GO_V(t') \mid t' \in \text{children of } (t)\} \text{ if } t \neq V \end{cases} \quad (1)$$

The semantic contribution factor is defined as c_e for the edge $e \in E_T$ linking to GO term t along its child term t' . Later calculating the GO – value for the GO term in DAG_V , the semantic value of GO term v , $SV_{go}(V)$ is defined as:

$$SV_{go}(V) = \sum_{t \in GO_V} GO_V(t) \quad (2)$$

For the two GO term given as, G and O , the semantic similarity between them is defined as:

$$SS_w(G, O) = \frac{\sum_{t \in S_G \cap S_O} GO_G(t) + GO_O(t)}{SV_{go}(G) + SV_{go}(O)} \quad (3)$$

In equation 3, the method proposed by Wang et al.⁴³ is used to compute the GO semantic similarity (SS_w). Moreover, $GO_G(t)$ is the GO – value of GO term t related to term t and $GO_O(t)$ is the GO of GO term t related to term O . S_Q is the set of GO terms including term O as well as all its ancestors.

The semantic similarity of the GO terms are calculated based on Best-Match Average (BMA)⁴⁴ strategy. The similarity is calculated of the terms those are associated with the influential markers and having an crucial impact on the particular pathway. This is defined as:

$$S_{BMA}(G1, G2) = \frac{\sum_{l=m}^i \max_{1 \leq n \leq j} S(go_{1m}, go_{2n}) + \sum_{l=n}^j \max_{1 \leq m \leq i} S(go_{1m}, go_{2n})}{i+j}$$

here, gene G1 annotated by GO terms set $GO1 = (go_{11}, go_{12} \dots go_{1i})$ and G2 annotated by $GO2 = (go_{21}, go_{22} \dots go_{2j})$.

Gene-gene Interaction

From the pathway semantic similarity calculation we have identified the top pathways which are showing high semantic values. To understand the connection of DLBCL pathways with the GBM related pathways we have performed an gene-gene interaction network (PPI) by using STRING database⁴⁵. The differentially expressed genes selected from "FindCluster" may have interaction under diverse cellular environment. Depending on this, we tried to understand the role of genes in in helping the pathways to cross the blood-brain barrier. The pathways found able to cross BBB are considered for further analysis.

Data availability

The three datasets of DLBCL patients used in this study are publicly available at GEO (<https://www.ncbi.nlm.nih.gov/geo/>). The detail information of the acquired datasets are as follows: 1st patient of DLBCL, GEO Accession No. GSM4579891 sample; 2nd patient of DLBCL, GEO Accession No. GSM4579892 sample; 3rd patient of DLBCL, GEO Accession No. GSM4579893 sample⁴⁶. The GBM dataset is downloaded from <https://www.10xgenomics.com/>.

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Author contributions statement

A.D. conceived and conducted the experiment. A.D., and U.M. analysed the results and drafted the manuscript.

Competing interests

The authors declare that they have no competing interests.

Supplementary Information

Supplementary Figure F1- A cell-type-specific pathway semantic similarity networks of Natural Killer cells.

Supplementary Table T1- The cell-specific pathways of the glioblastoma are reported in this table.

Supplementary Files

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