Transmembrane protein TMEM230, regulator of metalloproteins and motor proteins in gliomas and gliosis

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Contents

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Abstract

Glial cells provide physical and chemical support and protection for neurons and for the extracellular compartments of neural tissue through secretion of soluble factors, insoluble scaffolds, and vesicles. Additionally, glial cells have regenerative capacity by remodeling their physical microenvironment and changing physiological properties of diverse cell types in their proximity. Various types of aberrant glial and macrophage cells are associated with human diseases, disorders, and malignancy. We previously demonstrated that transmembrane protein, TMEM230 has tissue revascularization and regenerating capacity by its ability to secrete pro-angiogenic factors and metalloproteinases, inducing endothelial cell sprouting and channel formation. In healthy normal neural tissue, TMEM230 is predominantly expressed in glial and marcophate cells, suggesting a prominent role in neural tissue homeostasis. TMEM230 regulation of the endomembrane system was supported by co-expression with RNASET2 (lysosome, mitochondria, and vesicles) and STEAP family members (Golgi complex). Intracellular trafficking and extracellular secretion of glial cellular components are associated with endocytosis, exocytosis and phagocytosis mediated by motor proteins. Trafficked components include metalloproteins, metalloproteinases, glycans, and glycoconjugate processing and digesting enzymes that function in phagosomes and vesicles to regulate normal neural tissue microenvironment, homeostasis, stress response, and repair following neural tissue injury or degeneration. Aberrantly high sustained levels TMEM230 promotes metalloprotein expression, trafficking and secretion which contribute to tumor associated infiltration and hypervascularization of high tumor grade gliomas. Following injury of the central nervous or peripheral systems, transcient regulated upregulation of TMEM230 promotes tissue wound healing, remodeling and revascularization by activating glial and macrophage generated microchannels/microtubules (referred to as vascular mimicry) and blood vessel sprouting and branching. Our results support that TMEM230 may act as a master regulator of motor protein mediated trafficking and compartmentalization of a large class of metalloproteins in gliomas and gliosis.

1. Introduction

Glial cells provide physical and chemical support and protection for neurons and the extracellular compartment of neural tissue through secretion of soluble factors, insoluble scaffolds, and vesicles (Allen & Barres, 2009). Additionally, glial cells have regenerative capacity for remodeling their physical microenvironment. Glial cells also have the capacity for

changing the physiological properties of diverse cell types in their proximity. Various types of aberrant glial cells are associated with human disease, disorders, and malignancy (Allen & Lyons, 2018; Gao, Pan, Zhang, & Xia, 2023; Jakel & Dimou, 2017; Maiolo et al., 2021). Physical remodeling of the microenvironment is induced by glial cell microchanneling and scar formation. Physiological changes include glial cell remodeling of endothelial cell behavior for inducing tumor angiogenesis and remodeling of the immunomodulators of immune cells and the immune system.

Gliosis occurs in response to damage or insult to the central nervous system (CNS). This response involves the proliferation and epigenomic remodeling of glial cells, and cells (such as macrophages) affected by glial cells. As glial cells in gliosis may result in tissue remodeling and glial cell induced scar formation, gliosis historically was considered a detrimental reaction (Cieri, Villarreal, Gomez-Cuautle, Mailing, & Ramos, 2023; Iseki et al., 2012). For instance, the appearance of scars was thought to be a physical barrier for axonal and blood vessel regeneration. However, gliosis has more recently been thought to have also beneficial effects, such as limiting the physical extent of tissue damage by limiting the spread of cell damaging secreting factors, cytotoxic compounds, apoptotic and necrosis inducing factors to nearby cells or other compartments of the brain (Burda & Sofroniew, 2014, 2017; Burda, Bernstein, & Sofroniew, 2016). The physical barrier that inhibting circulation of tissue and cell damaging compounds is through scar forming scaffolds such as proteins and glycans.tion.

Glioblastoma (GBM) are the most aggressive tumors originating in the brain and contain heterogeneous and diverse infiltrating cell types. Higher grade astrocytoma, ependymoma, and oligodendroglioma are infiltrating tumors associated with GBM features. GBM may be derived from different tumor cell types including highly undifferentiated cell types such as stem cells or precursor/progenitor cells with an epithelial signature. This fact is essential to keep in mind when analyzing transcriptomic data from different patients with GBM, where the gliomas may originate from different cell types. For this reason, targeted treatments have been largely ineffective for GBM. In addition to glial cells, the glioma tumor tissue is composed of macrophages and other infiltrating immune cell types and blood vessel forming endothelial cells, that can also remodel the brain tissue. The aggressive behavior of these tumors is due to formation of highly circuitous, defective and permeable blood vessels and microchannels, formed by endothelial and phagocytic cells, respectively. Whether gliomas originate from and are maintained by tumor glial cells or other tumor cell types is

under intense investigation. Due to loss of the multifunctional properties as sensors and regulators of their microenvironment, glial cells are primary effectors in cancer or tissue remodeling following injury (Iv, Wintermark, & Massoud, 2019; Yamanaka, 2012). Gliomas are classified by tumor Grades (I-IV) based on their histology, cell morphology and the degree of aggressive infiltration potential. The tumors are often described as high- or low-grade gliomas, HGG, or LGG respectively (Bianconi et al., 2022; Yamanaka, 2012).

We previously demonstrated that human CNS glial cells have tissue revascularization and regeneration capacity by their ability to form microchannels and induce endothelial cell sprouting and blood vessel branching and formation by secreting pro-angiogenic signals. Additionally, glial cells regulate stress, immune, and cytotoxic response in their microenvironment (Cocola et al., 2021). These biological activities are modulated by glial intracellular trafficking and extracellular secretion through the endomembrane system. Trafficking of cargo is necessary for processes of endocytosis, exocytosis and phagocytosis mediated by cytoskeleton-based cargo movements using motor proteins. Trafficked components include neurotrophic factors, cytokines, glycolipids, glycoproteins, scaffolds, phagosomes, vesicles and organelles that regulate normal neural tissue homeostasis or immune response, inflammation and repair following neural tissue injury or degeneration (Cocola et al., 2021). Additionally, cell-to-cell (for instance, neural and endothelial cell) recognition and physical contacts, and cell-to-extracellular substratum recognition are mediated by scaffold components generated and trafficked in and secreted by the endoplasmic reticulum and the Golgi complex (Liu et al., 2021). The endoplasmic reticulum and the Golgi complex are the major hubs of the endomembrane system. Motor proteins (such as kinesins, dyneins and myosins) travel on microtubules (tubulin) and microfilaments (actin) using the intracellular generated energy (within the mitochondria) necessary for intra and extracellular movement (Ciocanel et al., 2022; Hassan Ibrahim, Balah, Gomaa Abd Elfattah Hassan, & Gamal Abd El-Aziz, 2022; Mayya et al., 2022; Obara & Kamura, 2022; Tang et al., 2022). Motor proteins are important in maintaining 3D structures of cells and tissue by trafficking subcellular components (factors, vesicles, phase condensate bodies, scaffolds, and organelles) to maintain or generate new intracellular dynamic cytoskeleton structures and new plasma and organelle membrane components. Diseases associated with loss of polarized basolateral-and apical cell polarity are often due to the formation of mesenchyme like cells generated by epithelial to

mesenchyme transition and with the loss of organization of tissue function and 3D structure (Guida et al., 2020; Carra et al., 2018; Grego-Bessa, Diez, Timmerman, & de la Pompa, 2004). Aggressive tumors such as gliomas are associated with loss of normal of tissue function, due to loss of the 3D tissue structure. Dynamic interactions of the intracellular cytoskeletons allow motor proteins and secreted metalloproteinases to generate microchannels or allow endothelial tip cells or immune cells to migrate and home. Microchannels and endothelial cell migration are part of normal (in wound healing) and pathological (metastasis) intracellular activities (Cocola et al., 2024).

Our research supports that misregulation of secretion processes or altered composition of the glial cell secreted components can promote aberrant physiological behavior of different cell types, including blood vessel endothelial and immune cells, in neural tissue through "at a distance" mechanisms (Carra et al., 2018; Cocola et al., 2021). Pathological intracellular and extracellular trafficking and secretion can be due to genomic mutations (such as in cancer), toxic environmental factors, or aberrant epigenomic events and processes (change in transcriptome) such as those induced by neural tissue injury, degeneration, or stress and by cytotoxic infectious agents, metals, or free radicals. Characterizing glial secreted factors, scaffolds, and vesicles in chronic or acute neural tissue injury or degeneration or cancer may provide new pathways for novel therapeutic intervention. For instance, a significant histopathological feature of aggressive tumors (GBM) is the formation of highly disorganized permeable blood vessels (Cocola et al., 2021). It is the loss of blood vessel structural integrity, endothelial cell-to-cell contacts, and regulated blood vessel permeability that contributes to the inability of the vessels to deliver therapeutic agents to the tumor mass or tumor cells. Current targets for anti-angiogenic therapies (targeted therapy such as anti-VEGF antibodies) provide minimal or no effect in increasing the overall survival of GBM patients (12–15 months following diagnosis). Characterization of glial mediated secretory and trafficking pathways are therefore an important goal for developing new and effective cancer, neural injury, and neurodegeneration treatments. Our research has identified TMEM230/C20ORF30 as a master regulator of glial and endothelial cell trafficking and secretion of intracellular factors, vesicles, scaffolds, organelles (endoplasmic reticulum, Golgi complex and mitochondria) and secretion of scaffold digesting enzymes. The exact mechanisms by which TMEM230 regulates trafficking is still not fully characterized. TMEM230 was found expressed in most cell types from zebrafish to human and its diverse functions are evolutionary

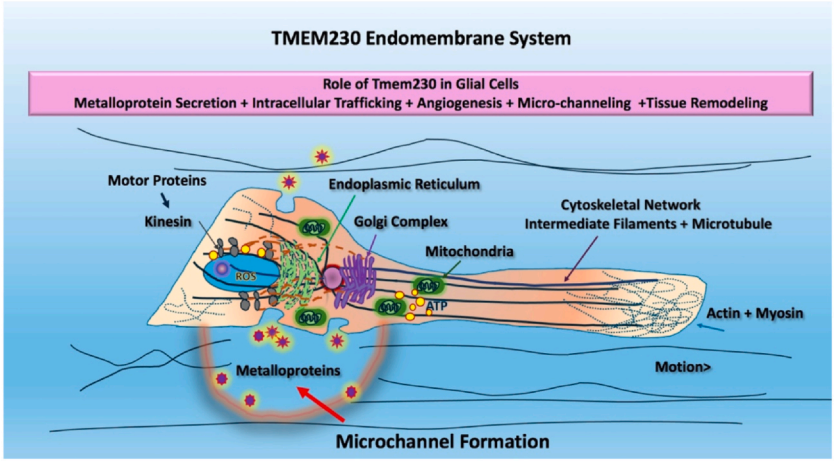

Fig. 1 TMEM230 is a component of the endomembrane system (endoplasmic reticulum, and the Golgi complex) regulating intracellular trafficking and secretion of cargo through scaffold motor protein mediated movement.

conserved in vertebrates (Carra et al., 2018; Cocola et al., 2021). TMEM230 is a membrane protein present in multiple membrane bound organelles of the cellular endomembrane and secretory systems. Forced change in TMEM230 expression in glial or endothelial cells results in modulation of expression of structural and functional genes of the endomembrane system, including genes encoding for motor proteins, secretory components, endoplasmic reticulum, and the Golgi complex (Fig. 1).

Modulation of TMEM230 expression critically influences 3D cytoskeleton structure and morphology and several diverse cell processes and behaviors associated with the endomembrane system. As expected, TMEM230 as an integral component of the endoplasmic reticulum and Golgi complex mediates glycan metabolism and glycoconjugation of diverse proteins and lipids, including components of metalloprotein complexes, trafficking, and secretion, described in this article. While our research supports that TMEM230 is an integral component of the endoplasmic reticulum/Golgi complex and regulator of trafficking and secretion, its role in glycobiological processes in cytoskeletal structure, motor protein force-drive movement, and metalloprotein activities is not well characterized. In terms of histopathology, our research endorses that aberrant high levels of the TMEM230 expression are instrumental in influencing tumor patient survival and the induction of an aggressive state

of glioma tumors due to the loss of proper regulation of motor protein facilitated cargo trafficking and metalloprotein complex formation.

TMEM230 expression and expression of certain classes of metalloproteins and motor proteins are linked as shown in this article (Fig. 1). Metalloproteins have many functions including sequestering essential or cytotoxic metals (Kunkle & Skaar, 2023), cellular metabolism (redox-active heme prosthetic group containing cytochromes), iron metabolism and regulation (six-trans-membrane epithelial antigen of the prostate protein (STEAP) family), (Lenoir, Rollason, Desmeules, & Samer, 2021) zinc finger nucleases (Barzegar & Pirouzpanah, 2023; Li, Quan, Ni, Li, & Qing, 2023; Liu, Liu, Lin, & Chen, 2023; Zhao, Wen, Zhang, Jiang, & Di, 2023), and zinc finger proteases that act in intracellular and extracellular tissue remodeling in cancer, stress, inflammation, wound healing and immune response (Almhjell & Mills, 2018; Garcia, Magalhaes, & Arruda, 2006; Hu, Chan, Sawyer, Yu, & Wang, 2014; Kunkle & Skaar, 2023; Nastri et al., 2019; Rouffet & Cohen, 2011; Wilson, Apiyo, & Wittung-Stafshede, 2004; Yang et al., 2016).

TMEM230 was initially identified in endothelial cells as being necessary to maintain normal blood vessel structural integrity and promote proper blood vessel network formation in diverse human tissues and in zebrafish early development (Fig. 2) (Carra et al., 2018; Cocola et al., 2021). Additionally, in zebrafish proper levels of TMEM230 was able to renormalize pathological and defective blood vessels. Further research supported that proper expression level of TMEM230 was necessary to maintain the morphology and 3D structure of

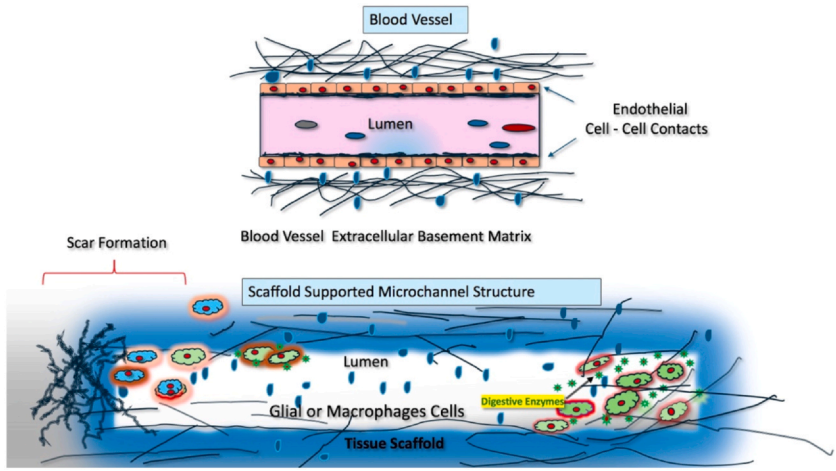

Fig. 2 TMEM230 regulates endothelial cells in blood vessel formation and glial cell microchanneling.

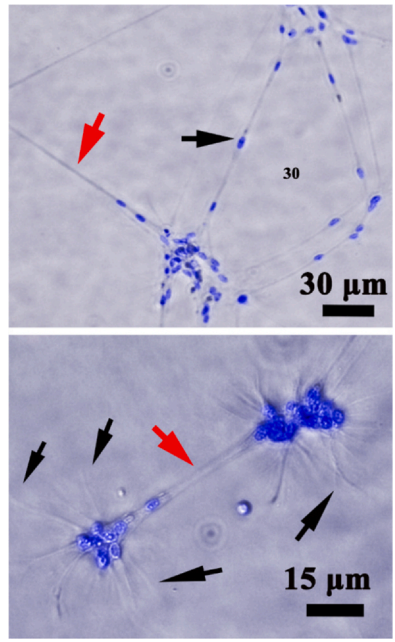

Fig. 3 TMEM230 promotes aberrant remodeling of the extracellular microenvironment by excessive microchannel formation and infiltration of glial cells by inducing secretion of metalloproteinases and scaffolds that enhance cell traction and migration. Red arrows, microchannels in which glial cells are migrating, black arrows glial infiltrating into 3D Matrigel. Blue is DAPI (4′,6-diamidino-2-phenylindole) fluorescent staining of glial cell nuclei.

glial cells, and their capacity for microchanneling and the homeostasis of their extracellular microenvironment (Fig. 2).

That TMEM230 has a role in gliomas was identified in tumor glial cells in which TMEM230 was upregulated. Upregulation resulted in aberrant remodeling of the extracellular microenvironment through excessive microchannel formation by infiltration of glial cells secreting metalloproteinases and scaffolds that enhance traction for cell migration (Fig. 3). Additionally, in co-culture assays of glial tumor cells and human umbilical vein endothelial cells (HUVECs), upregulation of TMEM230 in glial tumor cells promoted defective and highly permeable blood vessel formation and destructive tissue remodeling by uncontrolled blood vessel sprouting and infiltration into tissue (Fig. 2).

This supported that glial cells in addition to secreting metalloproteinases also secreted pro-angiogenic factors (Fig. 4).

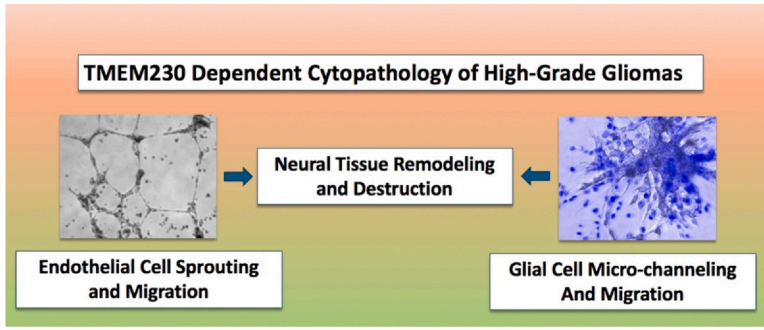

Fig. 4 The role of TMEM230 in cytopathology of high-grade gliomas. High levels of TMEM230 in endothelial cells or glial cells promotes highly permeable blood vessels and destructive tissue remodeling by uncontrolled blood vessel sprouting and infiltration and glial cell microchanneling.

The destructive tissue remodeling induced by metalloprotein synthesis and secretion is driven by highly active motor protein activities dependent on ATP synthesis in the mitochondria. Clinical therapeutic applications for downregulating TMEM230 and inhibiting tumor driven angiogenesis and microchanneling in aggressive and highly vascularized tumors have enormous potential. The current state of knowledge of the functional role TMEM230 in the pathogenesis of gliomas is presented here. As controlled regulation of TMEM230 expression is equally important for medical applications in wound healing, our results are equally relevant in gliosis, and acute or chronic neural injury/degeneration.

2. Aberrant high level of transmembrane protein, TMEM230 is associated with lower survivability of glioma patients

Expression analyses of published and open access mRNA sequencing datasets determined that TMEM230 was differentially expressed in tumor tissue cells from patients with low- or high- grade gliomas (Cocola et al., 2024) (Figs. 5 and 6).

The tumor patient gene expression analyses supported that TMEM230 has prognostic value as a tumor marker for aggressive high-grade gliomas since a higher level of *TMEM230* was associated with lower patient survivability (Figs. 5 and 6). Higher TMEM230 was also associated with worse prognosis. Moreover, it was observed that a higher percentage of patients died more rapidly compared to patients with lower expression of *TMEM230*.

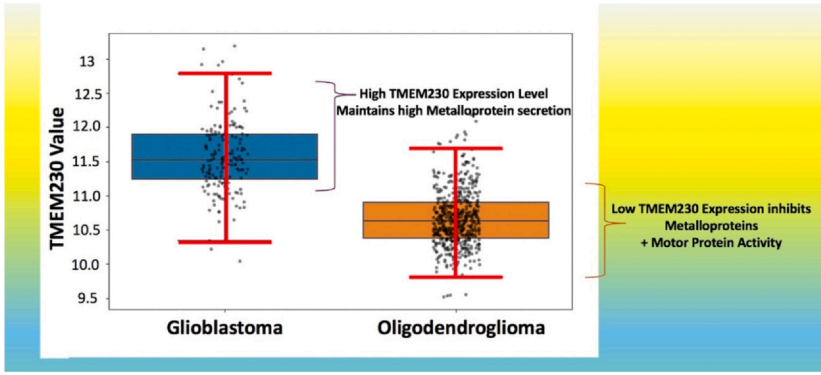

Fig. 5 Expression level of TMEM230 in oligodendroglioma and GBM. Glioblastoma multiforme tumors showed significantly elevated level of TMEM230 mRNA compared to oligodendroglioma (unpaired t-test $P < 0.0001$). Glioblastoma multiforme features may arise from glial neoplasms such as lower grade oligodendrogliomas due to upregulation of TMEM230 expression.

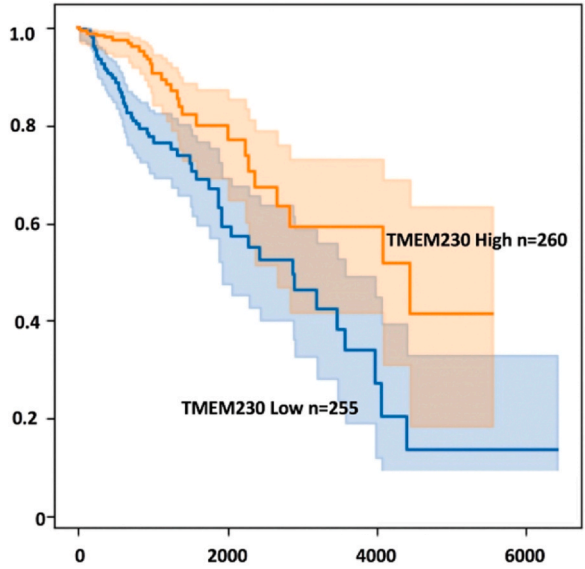

Fig. 6 Kaplan-Meier Curve. Log-rank test p-value between TMEM230 high and low = 0.0001324. Expression of TMEM230 in low- and high- grade gliomas analyzed from The Cancer Genome Atlas. Kaplan-Meier survival analysis correlated poor prognosis with high TMEM230 expression level. Vertical is probability of patient surviving, horizontal is number of days. The values are expressed in log2 of the number of normalized reads.

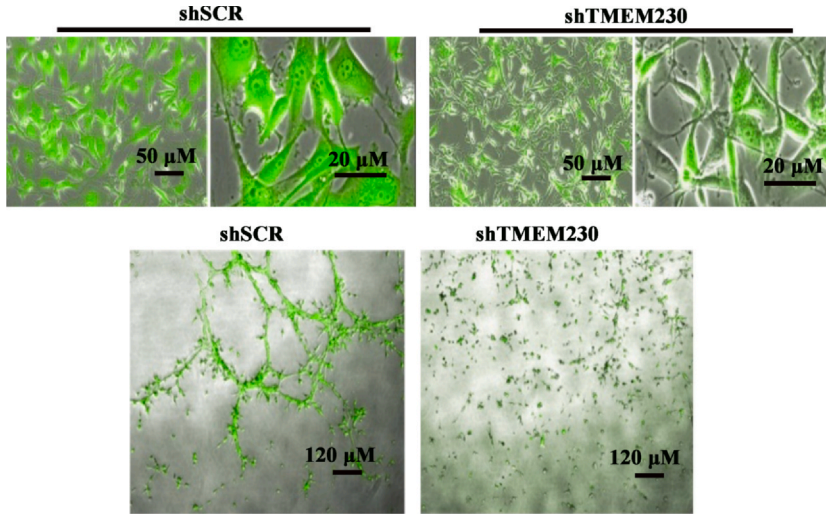

Fig. 7 Downregulation of TMEM230 promoted loss of U87-MG cell-substratum adhesion and microchannel formation. U87 control cells expressing endogenous TMEM230 (lentiviral construct expressing shSCR+eGFP) and U87 cells in which TMEM230 was constitutively downregulated (shTMEM230+eGPF) were cultured in 2D (upper panel) or 3D (lower panel) conditions. Cells in which TMEM230 were downregulated displayed decrease of cytoplasm volume and extensions in 2D cultured (upper panel) and inability to form microchannels and infiltrate in 3D cultures (lower panels). Cells were monitored at 24 and 48 h. Green: GFP (green fluorescent protein).

3. TMEM230 promotes glial cell growth, cytoskeleton structure, micro channeling and extracellular matrix remodeling

The pathological role of TMEM230 in gliomas was provided by the observation that TMEM230 expression was necessary to maintain tumor glial cell, U87-MG (a model of human GBM) growth and viability (Fig. 7, **upper left and right panels**). Downregulation of TMEM230 in U87-MG resulted in loss of cell-to-cell contacts, cell morphology and cell-to-substratum adhesion resulting in cell death **(upper right panel**). Loss cell morphology was evident by the appearance of reduced volume of the cytoplasm of glial tumor cells in which TMEM230 was downregulated (**right upper panel**). Loss of cell and cytoplasm morphology supported that TMEM230 was necessary for intracellular cytoskeletal and extracellular scaffold activity to maintain cell-to-substratum adherence.

Glial cell detachment from the substratum in adherent 2D cultures suggested that TMEM230 regulates and is part of the endomembrane

system necessary for intracellular shuttling of cargo between cell organelles for cell viability and secretion of scaffolds that support substrate adhesion. Both intracellular cytoskeletons and membrane components need to be regenerated for maintain cell morphology, adhesion, and tissue structure. Loss of these activities is associated with aggressive gliomas. This agrees with our previous studies that TMEM230 expression was also necessary for viability and maintaining morphology "normal" of cells. As TMEM230 was necessary for viability of normal and tumor cells, TMEM230 is a master regulator gene for cell viability, in agreement that endomembrane intracellular trafficking and secretion of cargo are essential processes for all eukaryotic cells. Candidate genes co-regulated or directly regulated by TMEM230 were evaluated in patient gliomas to better understand their role in tumor associated microchanneling and angiogenesis.

4. TMEM230, regulator of metalloproteins and motor proteins

Tissue culture studies (Fig. 7) supported that TMEM230 promoted tissue remodeling through formation of aberrantly induced microchannels and angiogenesis by regulating endomembrane organelle mediated secretion of scaffold digesting enzymes, phagosomes, and pro-angiogenic factors. Cell and tissue 3D morphology and cargo trafficking and secretion are powered by motor proteins on intracellular scaffolds. Motor proteins derive their enzymatic activity through ATP synthesis in mitochondria. To identify specific secreted genes, cellular components and pathways differentially expressed with TMEM230, transcriptomic analysis of approximately 200 patient samples with oligodendroglioma and a cohort of 172 patient samples with GBM from the The Cancer Genome Atlas (TCGA) RNA sequencing (RNAseq) database were were carried out (The Cancer Genome Atlas Research Network) (Fishbein & Wilkerson, 2018; Ganini et al., 2021). Analyses was performed using the TCGA2STAT R Package as previously described (Cocola et al., 2021). Transcriptomic analyses of oligodendroglioma and GBM patients were performed by correlating gene expressions of high from LGG patients with high or low TMEM230 expression (Tables 1–7, Tables 8–15).

Transcriptomic analysis supported that upregulation of TMEM230 regulated genes associated with the intracellular trafficking and secretory pathways via the endoplasmic reticulum and Golgi complex. Prominently,

Table 1 Motor proteins (Oligodendroglioma).

Down-regulated in TMEM230 high vs TMEM230 low		
Gene name	log2 fold change	padj
KIF13A	−0.645735746	6.2055E−10
KIF21B	−0.605161577	7.2921E−07
MYO7B	−0.807092208	1.8469E−05
MYO3B	−1.288598709	7.799E−06
KIF26B	−0.950382345	1.8139E−06
MYH6	−1.070831021	3.8505E−07
MYH7	−1.050918462	6.7854E−08
Up-regulated in TMEM230 high vs TMEM230 low		
Gene name	log2 fold change	padj
ACAA2	0.913329797	3.498E−12
DNAH5	0.839588708	9.879E−05
DNAH6	1.319053027	1.117E−09
DNAH9	0.950160895	4.55E−07
KIF17	0.821293394	4.665E−05
MYL12A	0.870387552	7.59E−14
DNALI1	1.114803575	2.262E−16
MYH11	1.581580932	1.199E−08
DNAH11	1.706396996	5.261E−09
DYNLT3	1.016444048	6.28E-08
KIF23	1.405722978	2.95E−08
MYO7A	3.489E-06	5.881E−05
MYO1D	0,899149606	3.489E−06
CENPE	1.031405117	5.672E−06
KIF18A	1.22867047	1.495E−06

(*continued*)

Table 1 Motor proteins (Oligodendroglioma). (*cont'd*)

Üp-regulated in TMEM230 high vs TMEM230 loq		
KIF16B	0.81260936	2.322E−09
MYO5B	0.92880452	4.433E−06
MYO3A	3.544206561	9.949E−10
MYO5C	0.670532751	7.61E−05
DYNLRB2	1.162422099	3.576E−08
KIF2C	1.462097304	1.347E−09
MYL9	0.905556738	2.883E−08
MYO1F	0.770518585	6.949E−08
MYO1G	0.90531234	9.8417E−05

Table 2 ER to Golgi transport vesicle membrane genes (Oligodendroglioma).

Down-regulated in TMEM230 high vs TMEM230 low		
Gene name	log2 fold change	padj
SEC31B	−0,684474853	4.332E−07

Up-regulated in TMEM230 high vs TMEM230 low		
Gene name	log2 fold change	padj
CD74	1.26612603	2.2995E−12
HLA-DRB5	1.47150262	3.5507E−09
HLA-G	1.04778631	5.933E−07
HLA-DPB1	1.16146261	3.6264E−09
HLA-DRA	1.28729486	9.144E−11
FOLR1	0.95679845	1.4023E−05
HLA-DQA2	1.47702829	1.5256E−06
HLA-DQA1	1.37275808	3.9701E−07
HLA-DQB2	1.7149909	7.4269E−07
HLA-DRB1	1.34977933	1.7124E−10
HLA-DPA1	1.1674359	2.4531E−09
HLA-DQB1	1.47177913	2.2819E−09

Table 3 Metal-thiolate (Oligodendroglioma).

Up-regulated in TMEM230 high vs TMEM230 low

Gene name	log2 fold change	padj
MT1A	1.44461685	7.2043E−06
MT1L	0.93696562	4.0032E−07
MT1M	1.06393038	9.1884E−08
MT3	0.75449947	7.9841E−05
MT1E	1.08498072	1.6929E−09

Table 4 Zins-finger LIM-type proteins (Oligodendroglioma).

Down-regulated in TMEM230 high vs TMEM230 low

Gene name	log2 fold chane	P adj
LIMD1	−0.7308964	3.689E−06
SCEL	−1.2612462	2.8461E−05
LHX3	−2.0939123	5.1793E−10
MICAL1	−0.7668273	1.4858E−07
LHX5	−1.4200835	2.5068E−05
LIMS2	−0.7063435	5.4007E−05

Up-regulated in TMEM230 high vs TMEM230 low

Gene name	log2 fold chan	P ad
TES	0.6761332	2.458E−05
LMO4	0.59023951	3.8884E−07
FHL2	1.14675265	6.7245E−08
FHL3	0.58172633	4.1221E−07
PRICKLE3	1.24245701	5.6638E−13
WTIPWTIP	0.67419197	3.8884E−07
CRIP1	1.00025008	3.7648E−07
PDLIM1	1.20860454	5.172E−09
CSRP1	0.73542364	3.261E−05

(*continued*)

Table 4 Zins-finger LIM-type proteins (Oligodendroglioma). (*cont'd*)

Up-regulated in TMEM230 high vs TMEM230 low		
TRIP6	0.84068716	1.8425E−09
MICALL2	0.59763839	3.412E−05
LMCD1	0.66784701	1.2554E−07
PDLIM4	1.78828601	3.8631E−15
PDLIM7	0.86391814	2.5426E−08

Table 5 Stress response gene (Oligodendroglioma).

Down-regulated in TMEM230 high vs TMEM230 low		
Gene name	log2 fold change	padj
HSF2BP	−0.908347	7.7465E−1
HSF4	−0.7251061	4.3617E-05

Up-regulated in TMEM230 high vs TMEM230 low		
Gene name	log2 fold change	P adj
PPP1R	0.62175825	3.755E−06
HSPB	1.75063591	1.133E−16
HSPA	1.658221773	2.599E−10
HSPB7	1.2016546	3.488E−12
FAM129A	1.054193278	1.852E−09
HSPB1	0.71757334	6.871E−05
HSF2BP	0.908347049	7.747E−12
HSPB11	0.680447962	3.058E−18
MAPK13	0.874961675	2.749E−05
GPR132	1.053802066	5.212E−11
RPS6KA1	1.08751337190866	2.154E−14
SERPINH1	1.013730287	8.817E−08
ANG	1.139260333	4.665E−14
HSPA1B	0.785635684	1.927E−05

Table 6 Inhibitor of metalloproteinase (Oligodendroglioma).

Up-regulated in TMEM230 high vs TMEM230 low

Gene name	log2 fold change	padj
C3	1.194274461	1.48614E−09
C4A	0.941894628	7.55799E−07
SFRP4	1.824971274	1.40E−15
FRZB	0.825891242	3.11864E−10
WFIKKN2	2.153416866	3.58057E−12
NTN5	0.702007619	7.43345E−05
PCOLCE	1.206972912	6.55E−09
TIMP1	1.890812165	1.32097E−14

Table 7 Metallopeptidases (Oligodendroglioma).

Down-regulated in TMEM230 high vs TMEM230 low

Gene name	log2 fold change	padj
ADAM22	−0.699444617	6.8986E−06
ADAM29	−1.250009	7.2835E−09
MMP16	−0.7481858	1.6841E−05
ADAMTS6	−0.6262533	5.9338E−05

Up-regulated in TMEM230 high vs TMEM230 low

Gene name	log2 fold change	padj
MMP7	3.30544251	1.7122E−10
ADAM33	0.9625628	1.5984E−06
ECE1	0.63492107	1.8681E−11
MMEL1	3.59923777	1.8792E−14
MMP9	2.37323283	5.4809E−10
PHEX	1.40403136	5.2483E−07
ADAMTS16	0.88427789	9.38E−05
ADAMTS4	1.06373676	7.4412E−06
MMP14	1.10187828	1.43E−08

(*continued*)

Table 7 Metallopeptidases (Oligodendroglioma). (*cont'd*)

Up-regulated in TMEM230 high vs TMEM230 low		
ADAM28	0.71220715	5.1928E−05
ADAMTSL14	0.96245365	5.6514E−05
ADAMTS3	1.5593766	2.87E−10
FAP	1.68585126	1.7131E−10
ADAMTSL4	0.7830829	2.4479E−06
ADAM12	0.9310504	7.6091E−05
ADAMTS7	1.2857075	1.8173E−08

Table 8 Kinesin Motor protein genes (GBM).

Down-regulated in TMEM230 high vs TMEM230 low		
Gene name	log2 fold change	padj
KIF14	−0.748144	4.2469E−06
KIF11	−0.7244394	3.1435E−07
CENPE	−0.6415596	6.2617E−05
KIF18B	−1,0530472	1.3887E−09
KIF26A	−1.2902956	8.0702E−08
KIF4B	−0.6958317	9.5823E−06
KIF5C	−0.6351271	3.0176E−05
KIFC1	−0.7595286	3.5448E−06
KIF4A	−0.5922348	4.722E−05
KIF13A	−0.8236063	1.9801E−16
KIF1B	−0.5982293	1.113E−09
KIF1A	−0.8784587	3.3646E−06
KIF21B	−1.4205933	3.3949E−12

Up-regulated in TMEM230 high vs TMEM230 low		
Gene name	log2 fold change	padj
KIF9	0.72062309	5.4054E−09

Table 9 Extracellular exosome or intracellular trafficking (GBM).

Down-regulated in TMEM230 high vs TMEM230 low

Gene name	log2 fold change	padj
RAB3B	−1.5832483	2.5999E−08
PCDH11X	−1.8397441	5.0158E−06
ALDH1L2	−0.5946679	2.6485E−05
EPHB1	−1.3773117	3.2861E−07
EYS	−0.9135857	4.6198E−05
TECTA	−0.8147011	1.7835E−08
SLC38A1	−0.83056459	1.2159E−05
BCR	−0.58399742	8.1735E−07
SFRP1	−1.22515891	8.106E−05
DAAM2	−1.09168586	1.0912E−05
ZNF711	−0.63932005	1.8253E−06
MASP2	−0.65786341	1.2469E−05
TNIK	−0.708542	3.8976E−08
SLC1A1	−0.90449334	2.7349E−05
PCDH15	−1.91022422	2.6365E−07
AGAP2	−1.38798601	2.4303E−05
NID1	−0.6219206	5.9604E−05
ATP6V0A4	−3.64478502	7.7753E−10
PROM2	−0.88849203	9.1998E−06
SPHKAP	−1.45428786	6.9319E−06
LRRN4	−1.65994908	3.3726E−05
MYO5A	−0.62966528	5.7273E−08
INHBC	−1.86223269	1.0216E−07
IGF2R	−0.61180186	6.5344E−06
PTPRD	−0.68541362	1.1827E−05

(*continued*)

Table 9 Extracellular exosome or intracellular trafficking (GBM). (*cont'd*)

Down-regulated in TMEM230 high vs TMEM230 low		
FASN	−0.81096257	9.9076E−14
SERINC5	−1.25886985	3.1473E−12
RYR1	−0.82161756	8.0453E−06
PTPRS	−0.71069064	2.6625E−09
NUMA1	−0.65188295	2.9131E−11
PKD1	−0.58720349	1.508E−05
SYNE2	−0.58512694	2.5865E−07
VPS13D	−0.62175775	4.3962E−09
GFRA1	−1.5010215	1.8253E−06
DNM3	−1.07001299	4.1672E−09
PPM1L	−0.68343447	1.5693E−07
ADCY1	−0.87164756	2.8404E−05
THSD4	−0.99233786	8.2493E−06
TTN	−0.65751081	9.8949E−05
NCAM1	−0.79316642	7.4446E−10
OPCML	−1.06199943	7.5661E−05
SPEN	−0.77557678	6.8559E−15
TAOK1	−0.73555451	9.9309E−06

Up-regulated in TMEM230 high vs TMEM230 low		
Gene name	log2 fold change	Padj
VPS29	0.6758223	4.1954E−13
NDUFA13	0.67472907	2.6542E−09
RPL34	0.61228351	1.7374E−08
HP	1.44645418	8.7818E−05
CIB1	0.6315761	7.5846E−13

HNMT	0.5889125	3.4263E−05
TXNDC17	0.7315454	3.6087E−13
RPS14	0.7606983	4.1596E−11
LGALS3	0.9738677	7.7063E−06
LGALS1	0.7038912	4.3817E−06
EFEMP1	1.0072322	7.246E−07
RPS19	0.6260068	8.9144E−08
SNRPD2	0.6859638	9.4249E−10
RPS18	0.7346911	5.3004E−07
TUBB1	0.7828019	3.6812E−07
RPS11	0.6157891	3.4344E−07
B2M	0.5884005	2.6609E−06
RPS10	0.6355744	7.5962E−07
RPS13	0.6405416	3.0198E−10
SKP1	0.6207732	1.8224E−12
RPS9	0.6445037	1.855E−09
IAH1	0.6543091	9.8816E−13
RPS7	0.6979611	6.1786E−08
RPS5	0.7654685	9.597E−08
IGFBP3	1.2246195	1.3534E−06
KRT7	1.2606473	5.912E−06
CMBL	0.6708503	2.8379E−11
RNASE3	1.0453002	6.1688E−05
SDCBP2	0.6050445	3.7814E−05
ATP5J2	0.8897034	1.7006E−14
SULT2B1	1.0130861	3.187E−05
PROCR	0.8585005	8.5653E−10

(*continued*)

Table 9 Extracellular exosome or intracellular trafficking (GBM). (*cont'd*)

Up-regulated in TMEM230 high vs TMEM230 low		
MYL6	0.7450174	1.8653E−11
AZGP1	1.2448184	2.5946E−05
SLPI	1.4783563	8.965E−06
RAB34	0.7547713	1.9314E−06
PSME1	0.6298171	1.355E−09
S100A6	0.834349	1.4995E−07
RPL24	0.6525794	1.6061E−07
RPL27	0.7267244	7.8371E−09
S100A4	1.1065039	4.9512E−08
SNRPE	0.7726581	2.2876E−08
RRAS	0.6953907	2.0872E−07
PHPT1	0.7221194	1.4514E−10
S100A8	1.095734	8.7191E−05
FTL	0.7413174	1.5768E−07
MGST3	0.6799954	3.9707E−12
COX7A2	0.6710769	2.1716E−07
ATP5H	0.6168093	1.8753E−14
ATP5O	0.6306962	1.4046E−10
ATP5I	0.6258153	7.8298E−11
MYL12B	0.6511174	1.085E−11
CYB5R1	0.8291807	1.57E−09
GNG10	0.6456537	3.546E−11
PRDX4	0.6726114	8.4906E−08
BLOC1S1	0.725038	5.1435E−11
PPCS	0.6693265	2.0134E−10

TSPAN6	0.6303802	2.2426E−09
S100A13	0.9256748	1.1554E−07
IGFBP7	0.8382349	3.8337E−08
IGFBP6	1.0403133	9.7654E−05
S100A11	0.7542408	1.0963E−06
ATP6V1F	0.6449142	1.9085E−10
S100A10	0.7999488	2.8342E−05
RARRES1	1.2592029	1.1406E−06
TNFSF13	0.6217417	1.1827E−05
DSTN	0.6012426	1.6382E−15
DERA	0.5911348	2.6297E−11
IFT20	0.7332656	4.4869E−12
RPS29	0.5991368	6.2745E−06
ARPC3	0.6198151	1.2356E−13
HLA-DRA	0.9309686	9.5009E−08
CRYL1	0.6496188	8.5404E−08
ANXA2P2	0.6672735	7.7462E−05
HIST1H4J	0.8959178	3.596E−05
NIT2	0.6203076	3.3975E−11
HLA-DRB1	0.762077	9.8764E−05
ITM2B	0.6260515	3.5659E−2
WFDC2	0.9786537	5.0158E−06
HEBP2	0.6175828	7.3026E−09
TRMT112	0.6160676	5.915E−14
VMO1	0.8625651	8.504E−07
HINT1	0.6730856	9.1898E−11
MB	1.1436162	5.6475E−05

(*continued*)

Table 9 Extracellular exosome or intracellular trafficking (GBM). (*cont'd*)

Up-regulated in TMEM230 high vs TMEM230 low		
CTSH	0.7373698	1.3944E−05
MDP1	0.6535805	4.5943E−10
CLIC1	0.6290122	4.3922E−07
CTBS	0.6045496	2.2089E−07
LYPLA1	0.7692383	6.3996E−13
GPX1	0.862515	1.5959E−12
ANXA2	0.6777246	5.1475E−05
GSTO1	0.9453972	7.2602E−12
ANXA4	0.6684204	9.0656E−08
IL18	0.7351453	4.6961E−06
NME2	0.6117188	3.5475E−10
USMG5	0.6161896	1.6481E−08
RPS3A	0.7874924	1.6717E−08
COMMD1	0.6889457	1.6974E−11
COMMD7	0.735904	6.8559E−15
NME1	0.5985207	7.3175E−07
VAMP8	1.0676493	7.8387E−1
DUSP23	0.7376824	3.4354E−06
NPC2	0.776493	3.4478E−08
CD48	0.8969478	1.6247E−05
VAMP5	1.1284088	1.5089E−13
RBKS	0.7026842	5.7564E−10
NDUFB9	0.6237464	1.0009E−08
CD63	0.7380292	1.9028E−10
C1QA	0.7320496	5.1909E−05

CSTA	0.8773855	2.541E−05
AHCY	0.5940091	1.7177E−11
C1S	0.8069595	8.9035E−05
NDUFB3	0.7198828	2.2078E−14
NDUFB1	0.8276873	1.9084E−13
GLRX	0.7511476	5.8544E−08
SRI	0.6526705	2.4514E−10
UQCR10	0.5975324	8.9592E−09
COX5B	0.7600717	8.0936E−14
CST6	1.4326202	7.747E−05
PSMA7	0.5804151	3.8253E−12
LILRA5	1.2925036	1.2047E−05
HLA-DMA	0.6113753	4.6257E−05
CLEC3B	0.9278317	9.8773E−06
RPS15A	0.6182568	2.3298E−07
PSMB3	0.6231831	7.1957E−11
GPA33	1.12915	4.8232E−06
RPS3	0.5843155	3.0886E−06
DNAJB9	0.5852481	4.1962E−08
CD58	0.8931401	9.4812E−12
NQO1	0.6168477	1.7285E−05
NDUFA4	1.0307223	1.8753E−14
KRT10	0.6088758	5.4888E−10
HSPE1	0.6216058	1.0068E−13
PDZK1IP1	1.3991316	8.9526E-06
PSMB8	0.5815849	1.5777E−05
MFAP4	0.9772385	8.1263E−06

(*continued*)

Table 9 Extracellular exosome or intracellular trafficking (GBM). (*cont'd*)

Up-regulated in TMEM230 high vs TMEM230 low		
APOC2	0.8959767	2.8713E−06
CAPZA2	0.7923054	3.4361E−12
APOC1	0.8644549	1.69E−06
MGP	1.4236814	2.4178E−09
FAS	0.7248454	1.4147E−05
CPVL	0.9617094	3.5195E−09
TPT1	0.8040878	2.9131E−11
RPL26L1	0.592299	1.1107E−10

Table 10 Oxidative Phosphorylation (GBM).

Down-regulated in TMEM230 high vs TMEM230 low		
Gene name	log2 fold change	padj
ATP6V0A4	−3.644785	7.7753E−10
Up-regulated in TMEM230 high vs TMEM230 low		
Gene name	log2 fold change	padj
NDUFB9	0.62374635	1.0009E−08
COX7B	0.87297199	2.3522E−13
NDUFA13	0.67472907	2.6542E−09
NDUFB7	0.69885357	2.0909E−08
NDUFB6	0.61527023	6.475E−09
NDUFA11	0.71730284	1.1422E−08
NDUFB5	0.85177567	1.2245E−14
NDUFB3	0.71988281	2.2078E−14
NDUFB2	0.58449424	1.6742E−10
ATP5J	0.62758428	4.7132E−13
NDUFB1	0.82768725	1.9084E−13

COX7A2	0.67107686	2.1716E−07
ATP5I	0.62581526	7.8298E−11
UQCR11	0.80282522	9.4181E−13
ATP5G3	0.61770707	5.1256E−09
UQCR10	0.59753236	8.9592E−09
ATP5H	0.61680934	1.8753E−14
ATP5O	0.63069624	1.4046E−10
COX5B	0.76007173	8.0936E−14
COX6A1	0.69152491	6.5175E−11
ATP5G1	0.62683855	1.2424E−08
COX7C	0.60856128	1.4594E−12
ATP5L	0.65727907	2.7404E−11
ATP5E	0.78650764	5.5847E−20
NDUFV2	0.60331376	4.2937E−11
ATP6V1F	0.64491419	1.9085E−10
ATP6V0B	0.70175381	1.2179E−10
NDUFA7	0.61219425	2.4178E−10
ATP6V0E1	0.69356206	5.5112E−14
NDUFA4	1.03072229	1.8753E−14
NDUFA3	0.6511756	1.856E−09
NDUFA2	0.87267142	3.9684E−14
NDUFA1	0.68910768	4.0521E−14
NDUFC1 SDHD	0.6339842 0.60479099	4.398E−14 6.303E−14
ATP5J2	0.8897034	1.7006E−14
COX7A2L	0.594432	2.1073E−09
UQCRQ	0.84673604	1.7378E−14
NDUFAB1	0.58736978	6.3302E−10

Table 11 Mitochondrial proton-transporting ATP synthase complex genes (GBM).

Up-regulated in TMEM230 high vs TMEM230 low

Gene name	log2 fold change	P adj
ATP5J	0.62758428	4.71324E−13
ATP5I	0.62581526	7.82985E−11
ATP5G3	0.61770707	5.12557E−09
ATP5H	0.61680934	1.87528E−14
ATP5G1	0.62683855	1.24238E−08
ATP5L	0.65727907	2.74036E−11
ATP5J2	0.8897034	1.70058E−14

Table 12 Motor proteins (GBM).

Down-regulated in TMEM230 high vs TMEM230 low

Gene name	log2 fold change	Padj
DNAH10	−0.719201327	5.14529E−06
DNAH8	−3.891485042	6.01498E−06
KIF14	−0.748143987	4.24685E−06
MYO5A	−0.629665275	5.72731E−08
KIF11	−0.724439426	3.14353E−07
DNM3	−1.070012994	4.16716E−09
CENPE	−0,641559638	6.26173E−05
KIF18B	−1.053047245	1.38866E−09
KIF26A	−1.290295623	8.07021E−08
KIF4B	−0.695831728	9.58233E−06
KIF5C	−0.635127098	3.01757E−05
KIFC1	−0.759528649	3.54484E−06
KIF4A	−0.592234784	4.72198E−05
KIF13A	−0.823606281	1.98005E−16

KIF1B	−0.598229286	1.11301E−09
KIF1A	−0.878458714	3.36459E−06
KIF21B	−1.420593327	3.39495E−12

Up-regulated in TMEM230 high vs TMEM230 low

Gene name	log2 fold change	Padj
DYNLT1	0.625070239	7.08561E−09
MYL12B	0.651117396	1.08501E−11
KIF9	0.720623091	5.40535E−09
MYL6	0.745017362	1.86526E−11

Table 13 Mithocondrial inner membrane Protein (GBM).

Down-regulated in TMEM230 high vs TMEM230 low

Gene name	log2 fold change	P adj
HERC2	−0.623696574	1.15982E−10
RHBDL3	−0.936916383	1.35405E−08

Up-regulated in TMEM230 high vs TMEM230 low

NDUFA13	0.674729065	2.65424E−09
MRPS15	0.743467812	4.90128E−13
COX7B	0.872971985	2.3522E−13
NDUFA11	0.717302837	1.14222E−08
MRPS12	0.583798042	3.65237E−08
COX6A1	0.691524914	6.51754E−11
MRPL34	0.694350228	8.41078E−12
ATP5G1	0.626838545	1.24238E−08
COX7C	0.608561283	1.45942E−12
MRPL33	0.647359485	1.67175E−10
LGALS3	0.973867746	7.7063E−06

(*continued*)

Table 13 Mithocondrial inner membrane Protein (GBM). (*cont'd*)

Up-regulated in TMEM230 high vs TMEM230 low		
HERC2	0.623696574	1.15982E−10
MRPS28	0.630090252	7.44461E−10
MRPS23	0.635048227	1.54681E−10
NDUFC1	0.633984202	4.39799E−14
SDHD	0.60479099	6,303E−14
ATP5J2	0.889703395	1.70058E−14
COX7A2L	0.594431999	2.1073E−09
TMEM126B	0.685161767	6.36471E−11
NDUFB9	0.623746352	1.00091E−08
NDUFB7	0.698853572	2.09086E−08
ATP5EP2	0.716156498	3.12675E−13
NDUFB6	0.615270231	6.47503E−09
MRPS36	0.599220717	2.91314E−11
NDUFB5	0.851775669	1.22445E−14
MRPS33	0.673495885	5.03793E−11
NDUFB2	0.584494241	1.67421E−10
ATP5J	0.627584278	4.71324E−13
NDUFAB1	0.587369776	6,3302E−10
ATP5I	0.625815255	7.82985E−11
UQCR11	0.802825221	9.41806E−13
UQCR10	0.597532361	8.95921E−09
ATP5H	0.616809343	1.87528E−14
HIGD1A	0.750671145	3.83171E−12
ATP5O	0.630696236	1.40459E−10
COX5B	0.760071731	8.0936E−14

MRPL13	0.702981872	6.77158E−12
TMEM70	0.686344164	5.3278E−12
MRPL54	0.88604218	2.5888E−12
ATP5L	0.65727907	2.74036E−11
MRPL11	0.596946372	9.45785E−11
C19orf70	0.735601078	7.01415E−12
RPS3	0.584315504	3.08865E−06
ROMO1	0.920221374	3.02669E−14
RHBDL3	0.936916383	1.35405E−08
NDUFV2	0.603313765	4.29371E−11
TIMM8B	0.692312891	1.1415E−08
DNAJC19	0.645553975	2.04334E−11
SURF1	0.603642561	1.02786E−09
NDUFA7	0.612194255	2.41779E−10
NDUFA4	1.030722293	1.87528E−14
NDUFA3	0.651175598	1.85602E−09
NDUFA2	0.872671419	3.96836E−14
NDUFA1	0.68910768	4.05209E−14
MRPL22	0.681214835	1.21441E−13
UQCRQ	0.846736041	1.73776E−14
NDUFAB1	0.587369776	6.3302E−10

various metalloproteinases and metalloproteins were identified differentially expressed, as were motor proteins, such as kinesins, dyneins. As biological processes are modulated by genes that are upregulated or downregulated, to provide a better understanding, specific genes are shown based on whether they were upregulated or downregulated with TMEM230 high expression (Tables 1–7, Tables 8–15). As genes are upregulated are down regulated, this suggests that some gene activities are promoted or inhibited by TMEM230. Genes may be co-regulated with TMEM230 or TMEM230 may directly regulate the expression of genes, these latter genes

Table 14 Microtubule associated protein (GBM).

Down-regulated in TMEM230 high vs TMEM230 low		
Gene name	Log2 fold change	P adj
NUMA1	−0.651882949	2.91314E−11
DNAH8	−3.891485042	6.01498E−06
KIF14	−0.748143987	4.24685E−06
KIF11	−0.724439426	3.14353E−07
KIF5C	−0.635127098	3.01757E−05
KIF13A	−0.823606281	1.98005E−16
KIF1B	−0.598229286	1.11301E−09
KIF21B	−1.420593327	3.39495E−12
KIF1A	−0.878458714	3.36459E−06
GTSE1	−0.719736564	5.19381E−06
APC2	−0.659734823	4.24839E−05
DNAH10	−0.719201327	5.14529E−06
SPAG5	−0.622314005	7.72752E−06
NAV1	−0.79623389	1.14735E−10
DNM3	−1.070012994	4.16716E−09
CENPE	−0.641559638	6.26173E−05
NIN	−0.585956582	2.88502E−09
KIF18B	−1.053047245	1.38866E−09
KIF4B	−0.695831728	9.58233E−06
INCENP	−0.640963712	8.35428E−11
KIF26A	−1.290295623	8.07021E−08
KIFC1	−0.759528649	3.54484E−06
KIF4A	−0.592234784	4.72198E−05
CENPJ	−0.808580121	1.38655E−07
MAPT	−0.705610221	7.5527E−06

Up-regulated in TMEM230 high vs TMEM230 low

Gene name	log2 fold change	Padj
TUBA1C	0.726939675	2.75927E−06
MAP1LC3A	0.588619938	6.03604E−07
TUBB1	0.782801923	3.6812E−07
DYNLT1	0.625070239	7.08561E−09
KIF9	0.720623091	5.40535E−09

Table 15 Genes co-regulated with TMEM230 high vs TMEM230 low in Oligodendroglioma and Astrocytoma.

Gene name	log2 fold change	Padj
RNASET2	0.66504711	1.96E−07 ASTRO
RNASET2	0.64673582	2.417E−07 ODG
		GBM NO
STEAP 1	1.2	1.69119−05 ASTRO
STEAP 2	1.7	1.29244E−16 ASTRO
STEAP 3	1.2	5.34833E−03 ASTRO
STEAP3	1.38686168	2.10857E−11 ODG

being target genes of TMEM230. This observation prominently suggests that the disease role of TMEM230 can be potentially mitigated by promoting or inhibiting their expression for targeted therapies. See Tables 1–7 for detailed list of genes modulated in oligodendroglioma patient samples with high TMEM230 expression level.

5. Transcriptomic analysis of patient derived oligodendroglioma

By analyzing oligodendroglioma patient sample mRNA expression, 7 and 24 motor proteins were downregulated and upregulated, respectively (Table 1).

All vesicle membrane genes associated with the endoplasmic reticulum to Golgi transport were upregulated except for SEC31B which was down regulated (Table 2).

All metal-thiolate genes were upegulated (Table 3).

Out of 20 *zinc finger LIM-type genes,* 6 were downregulated and 14 upregulated (Table 4).

Of stress *response genes, 2 were* downregulated and 14 *were* upregulated (Table 5).

Diverse inhibitors of metalloproteinases and metallopeptidases were identified with TMEM230 high expression (Tables 6 and 7).

Collectively, the patient oligodendroglioma transcriptomic analysis suggests that higher levels of TMEM230 in higher tumor grade of oligodendrogliomas (Fig. 6) were correspondingly associated with aberrant regulation of both metalloproteins and motor proteins.

6. Transcriptomic analysis of patient-derived glioblastoma

In contrast to lower tumor grade ODG patients (Fig. 5) in which TMEM230 levels were lower than high tumor grade ODG and GBM patients, highest TMEM230 expression levels were associated predoiminantly with highest expression of metalloprotein genes. This supports that TMEM230 functions in activating metalloprotein expression and activities such as metalloproteinases that contribute to GBM cancer features such as cell infiltration into tissue and formation of a highly permeable vascularized tumor tissue. Defective blood vessels likely contribute to the inability to deliver anti-cancer therapeutic agents to tumor cells. Forced down-regulation of TMEM230 in aggressive infiltrating gliomas may promote down-regulation of metalloproteins and their potential in tissue extracellular matrix and basement membrane remodeling.

Transcriptomic analysis of GBM patient samples based on high TMEM230 expression level indicated that all the *Kinesin motor proteins* genes were down regulated with KIF9 upregulated (Table 8).

From the *Extracellular exosome transport genes* (188 genes) 43 were downregulated while 145 were upregulated (Table 9).

Previously we showed that low grade gliomas (LGG) were correlated with lower expression of TMEM230 compared to GBM. The patient

sequencing analysis supports that high grade gliomas and GBM tumors are likely induced by loss of the ability of TMEM230 to properly regulate metalloproteins while TMEM230 retaining regulation of motor protein activities. While both high-grade gliomas and GBM provide low patient prognosis, GBM has unique histopathological features not observed with high grade gliomas making GBM tumors untreatable and highly aggressive in growth and neural tissue remodeling capacity. Our results support that the GBM aggressive behavior is due to aberrantly higher levels TMEM230 in GBM compared to high grade gliomas promoting mis-regulation of motor protein expression and mitochondrial activities such as oxidative phosphorylation.

This is indicated by the 39 genes associated with oxidative phosphorylation being downregulated (Table 10).

Mitochondrial proton-transporting ATP synthase complex genes were downregulated (Table 11), while 17 motor protein genes were downregulated and 4 upregulated (Table 12).

The contribution of TMEM230 in high grade glioma and GBM oncogenesis is summarized in Fig. 4. Collectively our results support that change in ATP biosynthesis and motor protein expression by aberrant levels of TMEM230 results in change in mitochondrial membrane composition likely due to role of TMEM230 in regulating intracellular trafficking and TMEM230 being an integral membrane protein of mitochondria. This is supported by the majority of the mitochondrial inner membrane proteins being upregulated (Table 13).

Most *microtubule associated* genes were downregulated (Table 14).

In support that TMEM230 is a membrane protein of the endosome system, we observed that TMEM230 expression is co-regulated with RNASET2, a gene that has both intracellular function (a lysosome and mitochondria resident RNase) and a secreted factor necessary to allow tissue clearing and remodeling by phagocytic cells, including glial and macrophage cells (Table 15). Additionally, TMEM230 expression was co-regulated with members of the STEAP (6-transmembrane epithelial antigen of prostate) family of metalloreductases that function in metal metabolism. The family consists of STEAP1, STEAP2, STEAP3, and STEAP4 that are unique to mammals and appear to be localized within the Golgi complex. The co-regulation of RNASET2 and STEAP suggests that TMEM230 is localized to multiple organelles including mitochondria and lysosome.

7. Discussion

While our patient gene expression analysis cannot indicate which cells are regulated by TMEM230, our glial cell tissue culture studies support that TMEM230 regulates important expression of metalloproteins in neural tumor formation. Our in vitro tissue culture assays support that TMEM230 promotes remodeling and micro channeling of neural tissue through secretion of metalloproteins and regulates intracellular skeletal scaffold activities in glial cells and secretion of extracellular scaffolds by glial cells. Both maintenance and remodeling of glial cell and neural tissue 3D morphology, cytoskeletal and extracellular matrix are by motor protein mediated shuttling of cell generated scaffolds. The remodeling of tissue induced by secreted metalloproteins is driven by highly active motor protein activities dependent on ATP synthesis in mitochondria regulated also by TMEM230. Gene expression analysis of patients with high or low grade oligodendroglioma or GBM in this article have identified specific metalloprotein gene that contribute to aggressive tumor features and may also represent targets for therapeutic intervention. The most common metals associated with proteins in human cells are copper, iron and zinc having diverse functions including regulating oxidative damage and oxygen transport. Mutations or loss of activity of metalloproteins promote diverse neurodegenerative diseases such as amyotrophic lateral sclerosis (Genin, Abou-Ali, & Paquis-Flucklinger, 2023; Gupta, Vagha, Dhingra, & Shirsath, 2023). For some neurodegenerative diseases whether glial cells or neurons are responsible for the pathology is still unknown and under investigation. Important metalloproteins include metallothioneins (MTs) that are necessary to maintain homeostasis of essential metals or regulate their cytotoxic effects in cells such as those associated with oxidative stress. MTs are a family of cysteine-rich, low molecular weight proteins localized to the Golgi complex. MTs bind physiological (zinc, copper and selenium) and xenobiotic metals. Heavy metals such as cadmium, mercury, silver, arsenic, and lead are elements found within an organism or not naturally produced or expected to have an essential cellular functional role. Therefore, MTs play roles in protection from metal toxicity, in metal detoxification, or oxidative stress and regulation and sequestering of essential or dietary metals such as copper and zinc.

In the mitochondria, cytochrome oxidase protein binds the O_2 between a coper and an iron to transfer electrons to the O_2 molecule. Copper containg proteins are also superoxide dismutates (SOD) that

catalyze the decomposition of superoxides by converting these to oxygen and hydrogen peroxide (Policar, Bouvet, Bertrand, & Delsuc, 2022). Superoxide is produced as an intermediary product of oxygen metabolism and if not removed causes damage to diverse cellular components including DNA, RNA and proteins and cell lipid membranes. SOD is an important antioxidant defense in nearly all living cells and is essential in preventing senescence or oncogenesis.

The metalloreductase family, STEAP is part of the Golgi complex was also identified as a potential gene co-regulated or regulated by TMEM230. STEAP3 converts iron from insoluble ferric ($Fe3^{+}$) to soluble ferrous (Fe^{2+}). Functions of the STEAP family of Golgi complex are largely unknown.

In addition to MTs, our sequencing analysis suggest that TMEM230 regulates diverse metalloproteins that function in the detoxification of xenobiotics in neural tissue. For instance, cytochromes are one of the most important detoxifiers in xenobiotics and was identified associated with TMEM230. Cytochromes (P450s or CYPs) a superfamily of enzymes containing heme as a cofactor that function in part as monooxygenases (Lenoir et al., 2021). Cytochromes are commonly found in liver but are also found in the CNS and have a protective role in preventing cancer development or neural degeneration such as in Parkinson's disease. Cytochromes in general are terminal oxidase enzymes in electron transfer chains, and like TMEM230 are primarily membrane-associated proteins found in endoplasmic reticulum and mitochondria. P450s potentially metabolize thousands of endogenous and exogeneous chemicals, suggesting that TMEM230 may regulate or co-regulate cytochromes, and therefore TMEM230 and TMEM230 regulated metalloproteins may have a very important central role in the metabolization of toxic compounds including drugs and products of endogenous metabolism, or potentially the synthesis, and breakdown of hormone like products or neurotrophic agents in neural tissue.

8. Conclusion

The Golgi apparatus is the hub of the endomembrane and secretory system that glycosylates and packages proteins into membrane-bound vesicles in intracellular and extracellular trafficking. As the nexus of the secretory, lysosomal, and endocytic pathways, the Golgi complex is essential for the regeneration and recycling of most cell molecules including cell membrane components and intracellular and extracellular scaffolds.

The tubular connections of the Golgi complex link stacks together, with localization and tubular connections maintained and cellular cargo trafficked by microtubules and motor proteins. Microtubule (MT) dependent transport is achieved through motor proteins such as dynein or kinesin and ATP generated in the mitochondria. The MT network plays an important role in maintaining cellular morphology, 3D tissue structure, and intracellular trafficking and secretion of Golgi packaged cargo (Chen, Xu, Wu, Soba, & Hu, 2023; Dumitru, Stoica, Covache-Busuioc, Bratu, & Cirstoiu, 2023; Khine & Sakurai, 2023; Liu et al., 2021; Peracchia, 2023; Tachikawa, 2023; Tang & Ginsburg, 2023; Vlad, Dumitrascu, & Dumitrascu, 2023; Zhang, Srivastava, & Zhang, 2023). The Golgi and MT network is essential in establishing cellular polarity and neurite like outgrowths observed in sprouting blood vessels and certain glial cells and neurons. MTs are important for the movement of mitochondria (Fu et al., 2023; Genin et al., 2023; Palma et al., 2023; Tian, Jiang, Zhou, & Zhang, 2023), lysosomes (Banushi & Simpson, 2022; Chauhan & Patro, 2023; Knupp, Pletan, Arvan, & Tsai, 2023; Mutvei, Nagiec, & Blenis, 2023; Patra, Patil, Klionsky, & Bhutia, 2023; Rudinskiy & Molinari, 2023; Xue, Zhang, & Li, 2023), peroxisomes (Li et al., 2023), and various other organelles such as endocytotic or exocytotic vesicles (Ahmadi, Abbasi, & Rezaie, 2024; Martirosyan et al., 2023; Ming-Kun et al., 2023), in establishing and maintaining the functionality of the endoplasmic reticulum. In conclusion, our research supports that aggressive highly vascularized and infiltrating tumors with lower patient survivability were associated with aberrant expression of metalloproteinases and motor proteins. Many of these genes are necessary for shuttling and secretion of tissue digesting enyzmes for infiltration and microchannel formation by glial and macrophage cells and inducing sprouting of endothelial cells. Many of the metalloproteins identified as co-regulated with TMEM230, like TMEM230 are also localized to the endomembrane system. supporting that TMEM230 regulates and is part of the endomembrane system of cells.

We identified that various metalloproteins (such as MTs) (Gao et al., 2022; Kwon et al., 2023), cytochromes and STEAP3 (Shi, Lei, Xiong, Du, & Shi, 2024; Song et al., 2023) have expressions that are coregulated and colocalized to the Golgi complex or mitochondria membranes with TMEM230. This supports that TMEM230 like the metalloproteins play a prominent role in the protection of cells against various types of stress response that are cytotoxic and/or DNA damaging such as oxidizing molecules.

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