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A molecular approach to Glioblastoma Multiforme

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ABSTRACT

Glioma is a tumor of the central nervous system that occurs in the glial cells, Which it surrounds and protects the nerve cells. Glioblastoma Multiforme (GBM) is the most common and malignant sub-type of gliomas that arises from star-shaped cells called "astrocytes", which constitute the supportive tissue of the brain. GBM are known to be heterogeneous in outcome with majority having a poor prognosis, thus there is an urgent need for novel therapeutic approaches. The detailed understanding of GBM is established by the combination of histopathology and genomic information of the tumor that aids in the best choice of Personalized Medicine. In this article, seven GBM patients are discussed who underwent tissue diagnosis as well as tumor molecular profiling; the significance of the genes and associated mutations/variations picked up in each individual.

Key words: Brain, Genes, Genetic testing, Glioblastoma multiforme, Glioma, Mutations, Personalized medicine

Introduction

Glioma encompasses all tumors that are thought to be of glial cell origin. Glioblastoma multiforme (GBM) is usually highly proliferative because the cells reproduce quite fast and is assisted by a large network of blood vessels (neovascularization). They are generally found in the cerebral hemispheres of the brain, but can also be found in other parts of the brain and spinal cord.

The average incidence rate of GBM in the USA is 3.19 per every 100,000 of the population, and the median age of diagnosis is 64 years with incidence higher in men. Many genetic and environmental factors have been studied in GBM, but the majority are sporadic, and no risk factor accounting for a large proportion of GBMs has been identified.[1]

Types of gliomas include astrocytic tumors (World Health Organization classification) astrocytoma Grades I, II (astrocytoma), III (anaplastic astrocytoma), and IV (glioblastoma or GBM), oligodendrogliomas, ependymomas, and mixed gliomas.

GBMs are biologically aggressive tumors that present unique treatment challenges due to localization of tumors in the brain; inherent resistance to conventional therapy; limited capacity of the brain to repair itself; migration of malignant cells into adjacent brain tissue; variably disrupted tumor blood supply which inhibits effective drug delivery; tumor capillary leakage, resulting in an accumulation of fluid around the tumor; (peritumoral edema) and intracranial hypertension; a limited response to therapy and the resultant neurotoxicity of treatments directed at gliomas.[2]

The standard treatment for newly diagnosed glioblastoma (Grade IV) is biopsy or surgical resection, depending on the location of the tumor, followed by the treatment with radiotherapy (RT) and chemotherapy (CT) (temozolomide). Because surgery, radiation and CT are unlikely to result in a prolonged remission of GBM tumors owing to their nature, researchers are investigating the use of novel treatments when the first line of therapy has failed.

Various prognostic and predictive biomarkers which are currently used in GBM include O6-methylguanine-methyltransferase (MGMT) promoter methylation status and isocitrate dehydrogenase (IDH) 1 and 2 mutation status. Leibetseder *et al* reported a high frequency of IDH1 mutations and MGMT promoter methylation among young adult patients with primary GBM with favorable outcome. MGMT promoter methylation is also a predictive marker for response to adjuvant CT and RT.

All the cases discussed here were referred to Dr. Amit Verma who runs a specialised clinic for Personalized Cancer Medicine at Max Cancer Center, New Delhi. Here, patients with GBM underwent molecular profiling, where their tumor(s) was tested for either of the two somatic mutation gene panels: (i) 48 (6 patients); (ii) 315 with 28 translocations (1 patient) genes that are shown to be important drivers in the process

Table 2: Summary of gene mutations identified

Case number	Histology	Gene(s) involved	Genomic loci	cDNA alteration	Protein change	Likely molecular subtype[++]	Actionable mutation	Targeted therapy available
1	GBM	*TP53*	Chr17:7578203C>T	c.646G>A	p.Val21Met	Proneural[a]	None	None
2	GBM	*PIK3CA*	Chr3:I78952088A>C	c.3143A>G	p.His1048Arg	Classical[b]	Yes	*PIK3CA* - Everolimus, temsirolimus, buprasilib
3	GBM	*EGFR, PTEN*	*EGFR* amplification PTEN – Deletion (6 loci)*		EGFR over expression Loss of PTEN expression	Classical[b]	Yes	*EGFR* – Afatinib, cetuximab, erlotinib, gefitinib, lapatinib, panitumumab *PTEN* -Everolimus, temsirolimus,
4	GBM	*VHL, PTEN, RB1*	*VHL*-chr3: 10183817C>T chr3: 10183836C>T *PTEN*-Chr10: 89717681G>A *RB1*-chr13: 49027241C>T chr13: 49037870A>G chr13: 49037937C>T	*VHL* – c.286C>T c.305C>T *PTEN*-c.706G>A *RB1*-c.1808C>T c.2110A>G c.2177C>T	*VHL* – p.Gln96* p.Pro102Leu *PTEN* - p.Asp236Asn *RB1* - p.Ala603Val p.Met704Val p.Thr726Ile	Classical[b]	Yes	*VHL* - Bevacizumab, sorafenib, *PTEN* -Everolimus, temsirolimus *RB1* - None
5	Anaplastic Astrocytoma Grade III	*EGFR, TP53*	*EGFR* –Chr7: 55249003_55249011dupCAGCGTGGA TP53-Chr17: 7577538C>T	*EGFR* – c.2301_2309dupCAGCGTGGA *TP53* - c.743G>A	*EGFR* –p.Ser768_Asp770dup *TP53* - p.Arg248Gln	Classical[b] or Proneural[d]	Yes	*EGFR* – Afatinib, cetuximab, erlotinib, gefitinib, lapatinib, panitumumab *TP53* - None
6	Gliosarcoma	No mutation identified	-	-	-	Neural[c] or Classical[b]	No	-
7	GBM	EGFR, PTEN, CDKN2A/B, BCORL1,SETD2, TERT	EGFR - amplification, D46N, EGFRvII, EGFRvIII PTEN - Tyr68Hist CDKN2A/B loss BCORL1- Gln1076fs*6 SETD2 splice site 5278-2A>T TERT promoter -146C>T			Classical	yes	EGFR: Afatanib, Cetuximab,Erlotinib, Gefitinib, Lapatinib, Panitumumab PTEN - Everolimus, Temsirolimus

[a, b, c, d]Refer Table 1 for more details. Case 3: PTEN – Deletion (6 loci)*, chr10: 89685304_89685432; chr10: 89711891_89712012; chr10: 89717515_8917638; chr10: 89717638:89717772; chr10: 89720709_89720843 and chr10: 89720843_89720967, Case 5 - Other VUS genes mutations*, *ATRX* - H475D, Q883R; *CREBBP* - P1279L; *GLI* – P1668R; *MYCL1* - A117T; *BRCA2* - A1996T; *FGFR2* - A16T, *HGF* - D552del, *TGFBR2* - T1551I, EGFR: Epidermal growth factor receptor, GBM: Glioblastoma multiforme, PTEN: Phosphatase and tensin homolog

Table 3: Gene Information

Genes	Mutations/Alterations	Relevant Information
EGFR	Amplification - *EGFR*vII, Amplification - *EGFR*vIII p.Ser768_Asp770dup p.Asp46Asn	*EGFR* gene is located on chromosome 7p12 region and the protein encoded by this gene is a member of the tyrosine kinase superfamily. The binding of growth factors ligands results in the activation of multiple downstream pathways controlling proliferation and survival (Siegelin and Borczuk, 2013)
PTEN	Deletion of 6 loci p.Asp236Asn Tyr68His	*PTEN* gene is located on chromosome 10q23 and codes for the PTEN protein which directly antagonizes PI3K signaling. It undergoes genomic loss, mutation, or epigenetic inactivation in 40-50% of gliomas, resulting in high levels of PI3K activity and downstream signaling (Koul, 2008)
VHL	P.Gln96* p.Pro102Leu	A tumor supressor gene that plays an important role in mammalian oxygen sensing pathway through the polyubiquitinylation of hypoxia-inducible factor. Tumors linked to *VHL* inactivation are often highly vascular and can overproduce angiogenic factors such as VEGF
RB1	p.Ala603Val p.Met704Val p.Thr726Ile	Retinoblastoma transcriptional corepressor 1is a tumor suprressor gene and is located on chromosome 13q14.1-q 14.2.pRB interacts with other proteins to influence cell survival, the self-destruction of cells (apoptosis) and differentiation
TP53	p.Val21Met p.Arg248Gln	TP53, otherwise called tumor protein 53or p53 is located in the nucleus of cells throughout the body where it attaches directly to the DNA. When DNA is damaged, p53 activates other genes to fix the damage. Gene location - Chromosome 10
PIK3CA	p.His1048Arg	The *PIK3CA* gene provided instructions for making the p110 α protein, which is a subunit of an enzyme PI3K. It is a catalytic subunit which helps in phosphorylation. Studies suggest that PI3K signaling may be involved in regulation of several hormones and play a role in the maturation of fat cells (adipocytes)
SETD2	Splice site 5278-2A>T	*SETD2* locayed on chromosome 3 is a tumor supressor gene. It is a histone methyltransferase that is specific for lysine-36 of histone H3, and methylation of this residue is associated with active chromatin and has been found to been associated with hyperphosphorylated RNA polymerase II
TERT	TERT Promoter 146C>T	Telomerase reverse transcriptase is a catalytic subunit of enzyme telomerase, which is responsible for the lengthening of the DNA strands. It is normally repressed in somatic cells, resulting in progressive shortening of telomeres
CDKN2A/B	Loss	Cyclin dependant kinase inhibitor 2A gene situated on chromosome 9q21.3 provide instructions for several studies, p16 (INK4a) and p14 (ARF) which are the most studied. These proteins help in cell cycle and p16 binds to CDK4 and 6 while p14 protects a different protein called TP53, hence prevent tumor formation
BCORL1	Gln1076fs*6	BCL6 corepressor –like 1 encodes for a transcriptional repressor that exhibits homology to BCOR, but unlike BCOR does not interact with BCL-6, rather functions as a transcriptional repressor with Class II histone deacetylases, and potentially BRCA1

EGFR: Epidermal growth factor receptor, TCGA: The cancer genome atlas, PTEN: Phosphatase and tensin homolog, PI3K: Phosphatidylinositol-3-kinase, VEGF: Vascular endothelial growth factor, SETD2: SET domain containing 2

Tables 2 and 3. The identified variation is located in the DNA binding region of *TP53* and is known to cause loss of transactivation potential of the protein.[5] Functional TP53 is required for TMZ mediated apoptosis, thus rendering the patient insensitive to the cytotoxic effects of TMZ.

Based on the Recursive Partitioning Analysis (RPA) criteria (based on the clinical parameters), the patient lies in Group 3 with a median survival of 70 weeks. However, the patient survived for over 48 months (>220 weeks) since diagnosis, suggesting a favourable tumor biology. This is supported by the fact that EGFR expression (IHC was carried out) and amplification (NGS) were absent, which are indicators of good prognosis. Further, based on the presence of aberrant TP53 expression (IHC was carried out) and somatic gene mutation (NGS), the patient can be classified into "Proneural Molecular Subtype" [Table 1]. The median overall survival of proneural subtype is reported to be 36-48 months,[6] thus explaining the patient's natural course (favourable).

Further, a study by Le Mercier *et al*.[7] showed no additional benefit of adding TMZ to RT versus RT alone in proneural subtype in comparison to the classical subtype of glioblastoma [Figure 1]. Thus, this calls into question the continuation of TMZ beyond progression. To conclude, molecular profiling may help to analyze and predict the prognosis and possible response to conventional therapy and management.

Therapeutic implications

i. Proneural molecular subtype with expected median overall survival 36-48 months as observed in the case
ii. No additional benefit of continuing TMZ beyond progression.

Case 2

A 68-year-old male who is a known case of left temporal GBM underwent sub-total resection and was on TMZ and RT. He was put on maintenance TMZ.

Clinical questions

1. What is the patient-specific tumor biology? Can we predict the treatment response?

2. What if the disease progressed while on treatment? What else can be done?

Molecular oncology approach

1. Somatic mutation 48 gene panel testing was carried out.

Result interpretation

The genomic alterations picked up are reported in Table 2.

The identified variation in *PIK3CA* gene (p.His10484Arg) is within the hotspot region and highly conserved kinase domain and has been previously reported in endometrial and breast cancers.

Based on the clinical parameters using RPA criteria, it was found that the patient lies in the poor prognosis group with median survival of 40 weeks. Further, mutation in the *PIK3CA* gene, which known to be genetically altered in primary glioblastoma with poor prognosis [Figure 2] and distinguishes from secondary glioblastoma with good prognosis. Thus, both the clinical and molecular assessment suggest poor prognosis.

This variation may lead to constitutional activation of the phosphatidylinositol-3-kinase *(PI3K)/AKT* survival pathway thus resulting in growth factor-independent proliferation and protection from cell death. Activation of the *PI3K* pathway is known to play a role in radioresistance in glioma.[8]

Therapeutic implications

i. Based on the mutation identified, we can subtype the GBM into "classical molecular subtype" with a median survival of 40 weeks (poor prognosis). The patient may benefit

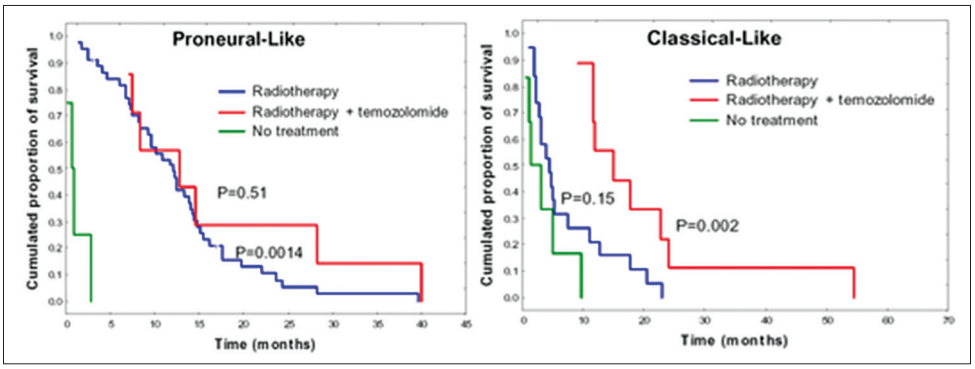

Figure 1: Predictive prognosis of Proneural subtype vs Classical subtype (Mercier et al)

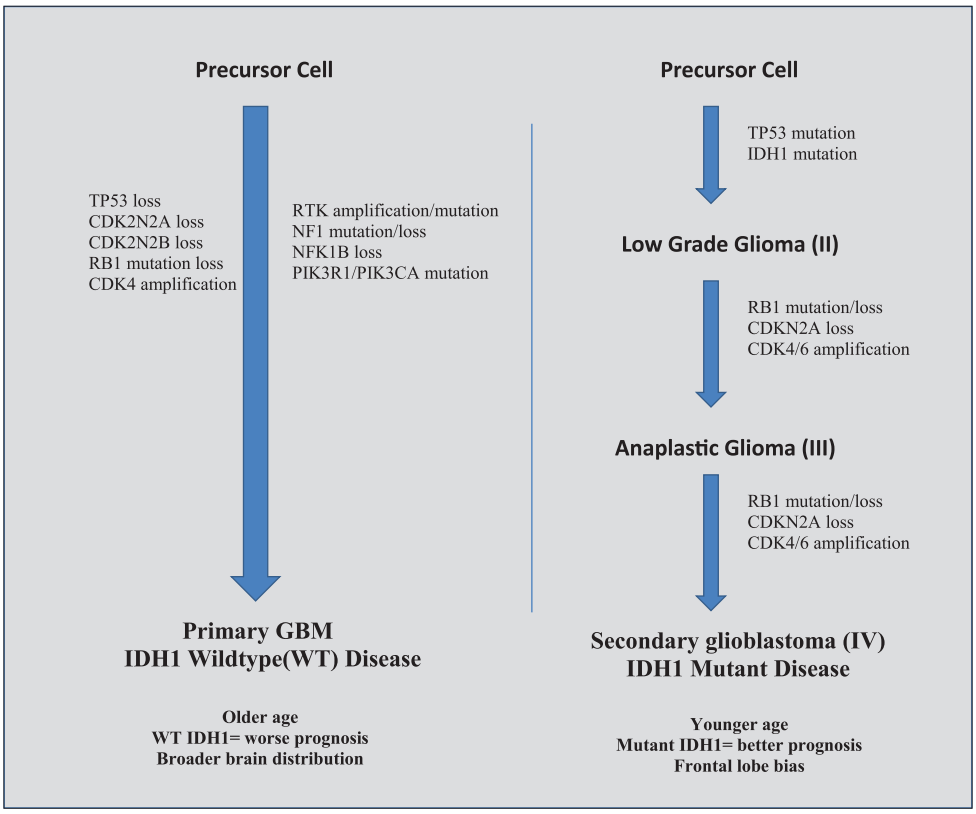

Figure 2: Stages in formation of Primary GBM vs Secondary GBM

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Appendix

List of 315 genes along with 28 translocations

ABL1	BRAF	CHEK1	FANCC	GATA3	JAK2	MITF	PCDC1LG2	RBM10	STAT4
ABL2	BRCA1	CHEK2	FANCD2	GATA4	JAK3	MLH1	PDGFRA	RET	STK11
ACVR1B	BRCA2	CIC	FANCE	GATA6	JUN	MPL	PDGFRB	RICTOR	SUFU
AKT1	BRD4	CREBBP	FANCF	GID4	KATBA	MRE11A	PDK1	RNF43	SYK
AKT2	BRIP1	CRKL	FANCG	GLI1	KDM5A	MSH2	PIK3C2B	ROS1	TAF1
AKT3	BTG1	CRLF2	FANCL	GNA11	KDM5C	MSH6	PIK3CA	RPTOR	TBX3
ALK	BTK	CSF1R	FAS	GNA13	KDM6A	MTOR	PIK3CB	RUNX1	TERC
AMER1	C11orf30	CTCF	FAT1	GNAQ	KDR	MUTTYH	PIK2CG	RUNX171	TERT (PROMOTER)
APC	CARD11	CTNNA1	FBXW7	GNAS	KEAP1	MTYDC	PIK3R1	SDHA	TET2
AR	CBFB	CTNNB1	FGF10	GPR124	KEL	MYC	PIK3R2	SDHB	TGFBR2
ARAF	CBL	CIUL3	FGF14	GRIN2A	KIT	MYCL	PLCG2	SDHC	TNFAIP3
ARFRP1	CCND1	CYLD	FGF19	GRM3	KLHL6	MYCN	PMS2	SDHD	TNFRSF14
ARID1A	CCDN2	DAXX	FGF23	GSK3B	KMT2A	MYD88	POLD1	SETD2	TOP1
ARID1B	CCND3	DDR2	FGF3	H3F3A	KMT2C	NF1	POLE	SF3B1	TOP2A
ARID2	CCNE1	DICER1	FGF4	HGF	KMT2D	NF2	PPP2R1A	SLIT2	TP53
ASXL1	CD27	DNMT3A	FGF6	HNF1A	KRAS	NFE2L2	PRDM1	SMAD2	TSC1
ATM	CD79A	DOT1L	FGFR1	HRAS	LMO1	NFKB1A	PREX2	SMAD3	TSC2
ATR	CD79B	EGFR	FGFR2	HAD3B1	LRP1B	NXX2-1	PPKAR1A	SMAD4	TSHR
ATRX	CDC73	EP300	FGFR3	HSP90AA1	LYN	NOTCH1	PRXCI	SMARCA4	U2AF1
AURKA	CDH1	EPHA3	FGFR4	IDH1	LZTR1	NOTCH2	PRKDC	SMARC91	VEGFA
AURKB	CDK12	EPHA5	FH	IDH2	MAGI2	NOTCH3	PRDSS8	SMO	VHL
AXIN 1	CDK4	EPHA7	FLCN	IGF1R	MAP2K1	NPM1	PTCH1	SNCAIP	WISP3
AXL	CDK6	EPHB1	FLT1	IGF2	MAP2K2	NRAS	PTEN	SOCS1	WT1
BAP1	CDK8	ERBB2	FLT3	IKBKE	MAP2K4	NAD1	PTPN11	SOX10	XPO1
BARD1	CDKN1A	ERBB3	FLLT4	IKIF1	MAP3K1	NTRK1	OKI	SOX2	ZBTB2
BCL2	CDKN1B	ERBB4	FOXL2	ILTR	MCL1	NTRK2	RAC1	SOX9	ZNF217
BCL2L1	CDKN2A	ERG	FOXP1	INNBA	MDM2	NTRK3	RAD50	SPEN	ZNF703
BCL2L2	CDKN2B	ERRF11	FRS2	INPP4B	MDM4	PAK3	RAD51	SPOP	
BCL	CKDN2C	ESR1	FUBP1	IRF2	MED12	PALB2	RAF1	SPTA1	
BCOR	CEBPA	EZHZ	GABRA6	IRF4	MEF2B	PARK2	RANBP2	SRC	
BCORL1	CHD2	FAM46C	GATA1	IRS2	MEN1	PAX5	RARA	STAG2	
BLM	CHD4	FANCA	GATA2	JAK1	MET	PBRM1	RB1	STAT3	

28 Gene rearrangements

ALK	FGFR3
BCL2	KIT
BCR	MSH2
BRAF	MYB
BRCA1	MYC
BRCA2	NOTCH2
BRD4	NTRK1
EGFR	NTRK2
ETV1	PDGFRA
ETV4	RAF1
ETV5	RARA
ETV6	RET
FGFR1	ROS1
FGFR2	TMPRSS2

List of 48 gene panel

ABL1	CSF1R	FGFR3	JAK2	NOTCH1	RET
AKT1	CTNNB1	FLT3	JAK3	NPM1	SMAD4
ALK	EGFR	GNA11	KDR	NRAS	SMARCB1
APC	ERBB2	GNAQ	KIT	PDGFRA	SMO
ATM	ERBB4	GNAS	KRAS	PIK3CA	SRC
BRAF	FBXW7	HNF1A	MET	PTEN	STK11
CDH1	FGFR1	HRAS	MLH1	PTPN11	TP53
CDKN2A	FGFR2	IDH1	MPL	RB1	VHL