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RAPID SHORT COMMUNICATION

Next-generation whole exome sequencing of glioblastoma with a primitive neuronal component

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Abstract

Glioblastoma with a primitive neuronal component (GBM-PN) was renamed from glioblastoma with primitive neuroectodermal tumor-like component (GBM-PNET) in the new WHO classification of tumors of the central nervous system in 2016. GBM-PN is a rare variant of glioblastoma. There were not so many publications on the investigation of GBM-PN. We did whole exome sequencing for 11 GBM-PN cases and found that the percentage of TP53, PIK3CA, PIK3R1, or PTEN mutation in our GBM-PN cases (72.7%, 27.3%, 27.3%, and 27.3% respectively) was much higher than that in cases in TCGA GBM 2008, TCGA GBM 2013, and TCGA lower-grade glioma databases. The findings indicate that GBM-PN is a distinct variant of glioblastoma. The next-generation sequencing can play a role in the diagnosis of GBM-PN especially for small biopsy cases. Eight out of 11 cases showed mutations in PTEN–PI3K pathway, which indicates that targeted therapeutic agents (PI3K inhibitors, mTORC1 inhibitors or dual PI3K/mTOR inhibitors) may be used for the treatment of GBM-PN in the future.

Keywords Glioblastoma with a primitive neuronal component (GBM-PN) · Next-generation sequencing · P53 · PIK3CA · PIK3R1

Introduction

Glioblastoma (GBM), WHO Grade IV astroctyoma, is the most common malignant primary brain tumor in adults. The majority of patients with GBM survive less than 18 months despite the combination of local radiation and temozolomide. Glioblastoma with a primitive neuronal component (GBM-PN) was previously named as "glioblastoma with primitive neuroectodermal tumor-like component (GBM-PNET)" and renamed in the new WHO classification of tumors of the central nervous system in 2016 [1]. GBM-PN is a rare variant of glioblastoma, representing approximately 0.5% of GBMs [2–4]. On histology, GBM-PN exhibits both infiltrating glial component, commonly astrocytic morphology, and primitive neuronal component, in which tumor cells have high nuclear/cytoplasmic ratio, salt-and-pepper chromatin, high mitotic and apoptotic rate.

In 2008, the first publication for GBM by the Cancer Genome Atlas (TCGA) Research Network reported the results of genomic and transcriptomic analysis of 206 GBMs, which included mutation sequencing of 600 genes in 91 of the samples [5]. The publication mainly focused on biologically relevant alterations in three core pathways, including p53, Rb, and receptor tyrosine kinase (RTK)/Ras/phosphoinositide 3-kinase (PI3K) signaling [5]. In 2013, the second publication for GBM by TCGA illustrated the landscape of somatic genomic alterations in more than 500 GBMs, especially emphasizing novel mutation and complex gene rearrangements in EGFR, PDGFRA and other signature receptors [6]. It was found that TERT promoter mutations correlated well with the increase of its mRNA expression in GBM [6]. In 2015, the publication for lower-grade gliomas by TCGA demonstrated genomewide analyses of 293 lower-grade gliomas. Lower-grade gliomas with an IDH mutation and 1p/19q co-deletion also had mutations in CIC, FUBP1, NOTCH1, and the promoter of TERT [7]. Lower-grade gliomas with an IDH mutation and the absence of

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1p/19q co-deletion most likely harbored mutations in TP53 (94%) and inactivation of ATRX (86%) [7]. Lower-grade gliomas without an IDH mutation had similar genomic aberrations and clinical behavior to primary glioblastoma [7].

Since GBM-PN is a rare tumor and is very difficult to diagnose on the initial frozen section, very few cases, if any, were included in the previous TCGA study. It is necessary to do the genomic analysis on this unique variant of GBM to fully understand this disease, which might open a new door for potential targeted therapies for GBM-PN. We described the morphological and immunohistochemical characteristics of GBM-PN in the previous publication [3]. In this paper, we did whole exome sequencing for 11 GBM-PN cases and analyzed common mutations in GBM-PN.

Materials and methods

Immunohistochemistry

Antigen retrieval was performed. Synaptophysin (Ventana, clone SP11, prediluted) or GFAP (Dako, clone 6F2, 1:300 dilution) was overlaid on original tissue sections and incubated overnight at 4 °C. Synaptophysin and GFAP immunohistochemical stains were performed on an automated immunostainer (Ventana Autostainer) with DAB Substrate. Immunohistochemical stains with appropriate positive and negative controls were blindedly evaluated by a neuropathologist and placed into one of two categories: positive or negative. Negative was defined as very weak-to-absent staining, or less than 25% strongly reacting tumor cells. Positive was defined as strong reaction in $\geq 25\%$ of tumor cells.

Genomic DNA isolation

IRB approval for this study was obtained from the Northwell Health Institutional Review Board. All formalin-fixed, paraffin-embedded (FFPE) tissue specimens (11 GBM-PN cases) were collected as part of standard clinical care and were considered to be leftover and unnecessary for patient treatment. Genomic DNA was extracted from tissue sections with both components of GBM-PN using QIAamp DNA FFPE Tissue Kit (Qiagen, Valencia, CA) as per the manufacturer's instructions. Genomic DNA samples were quantified using Qubit 2.0 Fluorometer (Life Technologies, Carlsbad, CA).

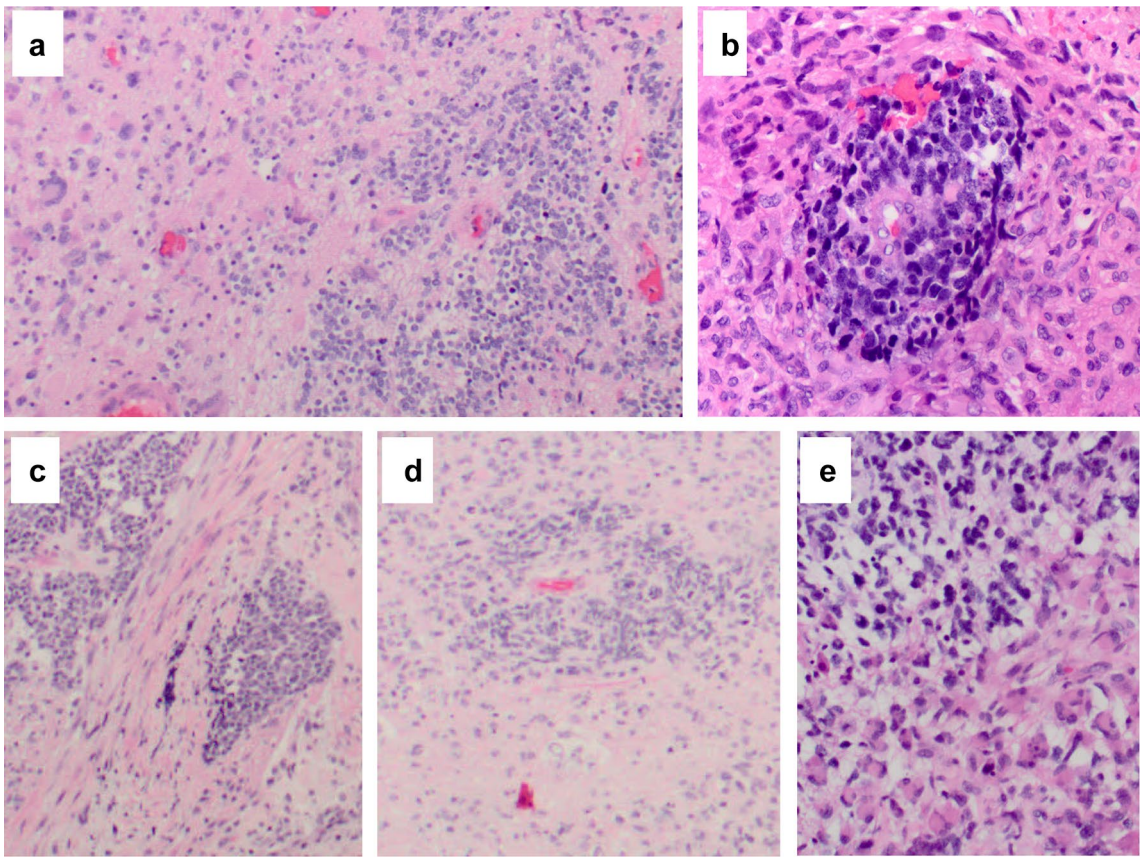

Fig. 1 Hematoxylin and eosin stain shows the astrocytic component in GBM-PN, and the primitive neuronal component in GBM-PN exhibiting high nuclear/cytoplasmic ratio and high mitotic activity. **a** Case 2. **b** Case 3. **c** Case 5. **d** Case 6. **e** Case 9

Next-generation whole exome sequencing

DNA library preparation and whole exome sequencing reactions were performed by Genewiz, Inc. (South Plainfield, NJ). Agilent SureSelect Target Enrichment System for Illumina Paired-End Sequencing (Agilent Technologies, Palo Alto, CA) and Illumina clustering and sequencing reagents (Illumina, San Diego, CA) were used during the experiment as per the manufacturer's instructions. The DNA libraries were validated using a High Sensitivity Chip on the Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA), and then quantified using both Qubit 2.0 Fluorometer (Life Technologies, Carlsbad, CA) and qPCR. After the samples were clustered on a flow cell using the cBOT, they were analyzed on the Illumina HiSeq 2500 instrument.

Data processing and mapping, and variant detection

Raw DNA sequence data generated from Illumina HiSeq 2500 were converted into fastq files and de-multiplexed with Illumina CASSAVA 1.8.4 program. Fastq files from each sample were imported into CLC Genomics Workbench 7.5.1. After sequence reads were trimmed to remove bases with low quality at ends, the sequence reads were mapped to Human Genome version 19 (hg19). Data coverage in the exon regions was evaluated and variant detection was analyzed using the quality-based algorithm with CLC Genomics Server program, using following parameters: minimum variant frequency = 5%; minimum coverage = 5; required variant count = 3. Finally, detected SNPs/INDELs were annotated with the dbSNP database, version 138, so the variants, which are present in dbSNP common database, were filtered out. We also used conventional GBMs as control to identified the unique alterations in GBM-PNET. These promising alterations were confirmed or eliminated by searching COSMIC

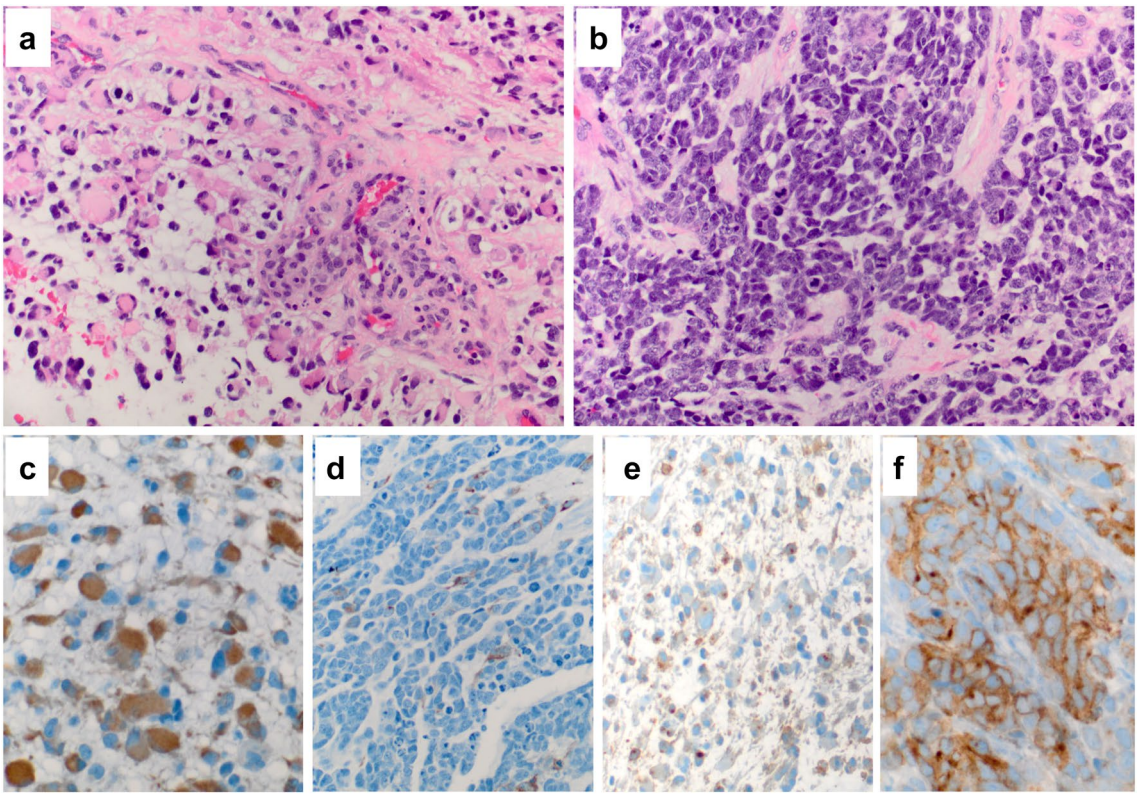

Fig. 2 The morphological and immunohistochemical staining profiles in GBM-PN. **a** Hematoxylin and eosin stain shows the astrocytic component in GBM-PN exhibits gemistocytoic morphology. **b** Hematoxylin and eosin stain shows the primitive neuronal component in GBM-PN exhibits high nuclear/cytoplasmic ratio and high mitotic activity. **c** Immunohistochemical stain demonstrates that GFAP is positive in the astrocytic component in GBM-PN. **d** GFAP is negative in the primitive neuronal component in GBM-PN. **e** Immunohistochemical stain demonstrates that synaptophysin is negative in the astrocytic component in GBM-PN. **f** Synaptophysin is strongly and diffusely positive in the primitive neuronal component in GBM-PN

Table 1 Gene mutations in GBM with a primitive neuronal component

Gene	Mutation (CDS)	Mutation (amino acid)	Mutation ID (COSM)	Mutation type	Case no
TP53	c.380C > A	p.S127Y	COSM43970	Substitution—missense	Case 1
TP53	c.836G > A	p.G279E	COSM43714	Substitution—missense	Case 2
TP53	c.711G > A	p.M237I	COSM10834	Substitution—missense	Case 3
TP53	c.1024C > T	p.R342*	COSM11073	Substitution—missense	Case 4
TP53	c.713G > A	p.C238Y	COSM11059	Substitution—missense	Case 4
TP53	N/A	N/A	N/A	N/A	Case 5
TP53	c.422G > A	p.C141Y	COSM43708	Substitution—missense	Case 6
TP53	c.482C > A	p.A161D	COSM11323	Substitution—missense	Case 7
TP53	No mutation	N/A	N/A	N/A	Case 8
TP53	c.578A > T	p.H193L	COSM11066	Substitution - Missense	Case 9
TP53	No mutation	N/A	N/A	N/A	Case 10
TP53	c.437G > A	p.W146*	COSM43609	Substitution—nonsense	Case 11
PIK3CA	c.278G > A	p.R93Q	COSM86041	Substitution—missense	Case 1
PIK3CA	c.1633G > A	p.E545K	COSM763	Substitution—missense	Case 6
PIK3CA	c.311C > G	p.P104R	COSM747	Substitution—missense	Case 11
PTEN	c.740_741insA	p.L247fs	N/A	Insertion-frameshift	Case 2
PTEN	c.322C > T	p.L108F	COSM4967205	Substitution—missense	Case 10
PTEN	c.850G > T	p.E284*	COSM28905	Substitution—nonsense	Case 11
PIK3R1	c.1783_1784delAAins	p.N595fs	NA	Insertion-frameshift	Case 2
PIK3R1	c.1784A > T	p.N595I	COSM3994405	Substitution—missense	Case 2
PIK3R1	c.1740C > A	p.Tyr580*	NA	Substitution—nonsense	Case 7
PIK3R1	c.328delC	p.Gln110fs	NA	Deletion-frameshift	Case 7
PIK3R3	c.139C > T	p.Arg47*	NA	Substitution—nonsense	Case 7
PIK3R4	c.1759G > A	p.Asp587Asn	NA	Substitution—missense	Case 2
PIK3CB	c.61G > A	p.V21I	NA	Substitution—missense	Case 4
PIK3CB	c.61G > C	p.D21H	COSM581887	Substitution—missense	Case 4
RB1	c.958C > T	p.R320*	COSM891	Substitution—nonsense	Case 4
ATRX	c.3973delC	p.His1325fs	NA	Deletion-frameshift	Case 1
CDKN2A	c.238C > T	p.R80*	COSM12475	Substitution—nonsense	Case 7

v74 (http://cancer.sanger.ac.uk/cosmic) or Ensembl (http://useast.ensembl.org/index.html).

Results and discussion

Only 2 out of 11 cases of GBM-PN were from previous study (those two cases are negative for IDH1 mutation) [3]. The rest of nine cases were primary glioblastomas. The median age of the 11 patients was 50 years (Ranging from 43 to 82 years). The male to female ratio was 3:1. Six cases occurred in the temporal lobe, two cases in the parietal lobe, one in the frontal lobe, one in the fronto-parietal region and one in the occipital lobe. On hematoxylin and eosin stained slides, GBM-PN is composed of the infiltrating glial component, commonly astrocytic morphology, and the primitive neuronal component exhibiting high nuclear/cytoplasmic ratio, salt-and-pepper chromatin, high mitotic and apoptotic activity (Fig. 1). All GBM-PN cases were selected by synaptophysin and GFAP immunostains. Synaptophysin and GFAP immunohistochemical stains were routinely performed on the original blocks of all GBM-PN cases before this project was started. GFAP was positive in the astrocytic component in GBM-PN, but was negative in the primitive neuronal component in GBM-PN. The primitive neuronal component of GBM-PN was positive for synaptophysin, but was negative for GFAP (Fig. 2 from case 4).

These frequently mutated genes including TP53, PIK3CA, PIK3R1, PIK3R3, PIK3R4, PIK3CB, RB1, ATRX, CDKN2A, EGFR, IDH1 and IDH2 were searched in the DNA sequence data from 11 GBM-PN cases (Table 1). Nine out of 11 GBM-PN cases had TP53 mutations (p.S127Y, p.G279E, p.M237I, p.R342*, p.C238Y, p.C141Y, p.A161Dp, H193L or p.W146* for individual positive case). Three out of 11 GBM-PN cases harbored PIK3CA (p.R93Q, p.E545K or p.P104R for individual positive case). PIK3R1 mutations (p.N595I, p.Tyr580*, p.Gln110fs) were identified in 3 out of 11 GBM-PN cases. PIK3R3 p.Arg47* mutation,

PIK3R4 p.Asp587Asn mutation, or PIK3CB p.D21H was noted in one GBM-PN case. RB1 p.R320* mutation presented in one case. ATRX p.H1325fs was seen in one case. One case had CDKN2A p.R80. There were no EGFR, IDH1 and IDH2 mutation in any of cases. None of cases in this study had previous history of low-grade glioma. The presence of IDH1 mutation in GBM-PN was only seen in the secondary GBM-PN cases [3].

We went to Memorial Sloan-Kettering Cancer Center cBioPortal for Cancer Genomics website (http://www.cbioportal.org/index.do) and searched the percentage of gene mutations for above genes in TCGA GBM and lower-grade glioma databases [8, 9]. We found that the percentage of TP53, PIK3CA, PIK3R1, or PTEN mutation in our GBM-PN cases (72.7%, 27.3%, 27.3%, 27.3% respectively) was much higher than that in cases in TCGA GBM 2008 (15%, 2.9%, 4.4%, 14.1% respectively), TCGA GBM 2013 (10.2%, 4.5%, 5.3%, 11.4% respectively), and TCGA lower-grade glioma (27.5%, 4.5%, 2.6%, 2.5% respectively) datasets [8, 9]. The above findings demonstrate GBM-PN is a distinct variant of glioblastoma at the molecular level.

Eight out of 11 cases showed mutations in PTEN–PI3K pathway (Table 2). PTEN was a negative regulator of PI3K signaling, which is a main regulator of cell growth and metabolism, cell survival, proliferation, angiogenesis, invasion and metastasis. PI3K-mediated PIP_3 production activated the canonical AKT-mTORC1 pathway. The presence of mutations in PTEN–PI3K pathway indicates that targeted therapeutic agents (PI3K inhibitors, mTORC1 inhibitors or dual PI3K/mTOR inhibitors) might be used for the treatment of GBM-PN in the future.

GBM-PN is an unique variant of glioblastoma at molecular level with high frequency of TP53, PIK3CA, PIK3R1, or PTEN mutation. It may be worth trying PTEN–PI3K pathway targeted therapies for the patients with GBM-PN.

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Compliance with ethical standards

Conflict of interest There is no conflict of interest.

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