Original Article

Glioblastoma with PNET-like components has a higher frequency of isocitrate dehydrogenase 1 (IDH1) mutation and likely a better prognosis than primary glioblastoma

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Abstract: Glioblastoma with primitive neuroectodermal tumor-like components (GBM-PNET), a rare variant of glioblastoma, poses both diagnostic and therapeutic challenges. Ten patients with GBM-PNET were investigated with a median age of 51.5 years and the male to female ratio of 4:1. The majority of patients (7 out of 10) showed ring-enhancing lesions on magnetic resonance imaging (MRI), which is classic for GBMs. Restricted diffusion was noted in 7 cases where diffusion weighted imaging (DWI) was performed, which correlates with the presence of PNET-like components. CD56 and vimentin immunostaining made the diagnosis of GBM-PNET much easier. Vimentin strongly and diffusely highlighted the astrocytic components and was negative in PNET-like components, while CD56 was strongly and diffusely positive in both astrocytic and PNET-like components. Seven out of 9 cases were positive for p53 in both astrocytic and PNET-like components. Two out of 8 cases harbored isocitrate dehydrogenase 1 (IDH1) R132H mutation, while IDH2 R172 mutations were not identified. Three out of 10 patients had a median survival time of 17 months while the two patients, whose tumor carried IDH1 mutation, were still alive after 15 and 31 months of follow-up. Compared to primary GBMs, GBM-PNETs might have a better prognosis. Further large scale studies are necessary to confirm this observation.

Keywords: Glioblastoma with PNET-like components, isocitrate dehydrogenase 1 (IDH1), glioblastoma, vimentin, CD56, diffusion weighted imaging (DWI)

Introduction

Glioblastoma (GBM), or World Health Organization (WHO) grade IV astrocytoma, is the most common malignant primary brain tumor in adults. The histopathology is heterogeneous [1], and the prognosis is grave despite more recent advances in treatment that typically includes local radiation combined with alkylating chemotherapy, such as temozolomide. Recently, isocitrate dehydrogenase 1 (IDH1) and 2 (IDH2) mutations have been observed in 80-100% of astrocytomas, oligodendrogliomas, oligoastrocytomas, and secondary glioblastomas, where they confer a significantly improved prognosis [2-5]. By contrast, IDH1 and IDH2 mutations only rarely occur in primary glioblastomas, other common brain tumors, and reactive gliosis [2-5].

Supratentorial CNS primitive neuroectodermal tumor (sPNET) is a primitive, embryonal malignant neoplasm, phenotypically recapitulating the primitive developmental stages of the central nervous system (CNS). It is more common in the pediatric population and is rare in adults [6-8]. Preoperative magnetic resonance imaging (MRI) of sPNETs frequently shows restricted diffusion on diffusion weighted imaging (DWI) [9].
A variety of neuroendocrine, neuronal, and glial immunohistochemical markers, such as synaptophysin, neuron specific enolase (NSE), neurofilament proteins, and glial fibrillary acidic protein (GFAP), are often demonstrated in sPNETs in pediatric population and are generally interpreted as evidence of differentiation along the neuronal or glial lineage. Occasional entrapped reactive astrocytes, however, can also be highlighted by GFAP and must be distinguished from genuine expression by tumor cells. A recent study has showed that adult sPNETs are different from pediatric ones. Adult sPNETs do not show astrocytic differentiation, which are negative for GFAP and partially positive for vimentin in 5 out of 12 cases [10]. sPNETs exhibit a very high MIB-1 (Ki-67) labeling index. Mutations in IDH1 have been observed in a small percentage of adult sPNETs [11, 10].

It is rare to see a primary brain tumor with both astrocytic differentiation and PNET-like features. Most of those documented in the literature have appeared as single case reports under a variety of names, such as (i) unusual variants of GBM/gliosarcoma (GS); (ii) PNET of the CNS with prominent glial differentiation; or (iii) malignant or high-grade glioneuronal neoplasms, not otherwise specified [12]. Rarely, cases have been defined as GBM with PNET-like component (GBM-PNET) [13-20, 12]. In a recent series of 53 cases of malignant glioma-PNETs, Perry et al. [12] reported N-myc or c-myc gene amplification in the PNET-like component of 43% of tumors, whereas common genetic alterations, typically associated with gliomas, were present in both glial and PNET components. CD56, also known as neural cell adhesion molecule (N-CAM), is a homophilic binding glycoprotein expressed on the surface of neurons and glial cells. Both GBM and medulloblastoma express CD56 [21-23]. Vimentin is an intermediate filament and is widely and strongly expressed in GBMs [21, 24]. The diagnosis and differentiation of GBM from sPNET and GBM-PNET is particularly challenging for the neuropathologist. Such distinction is of paramount importance as the treatments are different. Since sPNETs have a high risk to spread through cerebrospinal fluid (CSF), treatment protocols typically include craniospinal irradiation and platinum-based chemotherapy.

In this paper, we report 10 patients with GBM-PNET and demonstrate the important use of DWI, and immunohistochemistry for vimentin and CD56 in the diagnosis of these cases. We also document the presence of IDH mutation in these tumors and discuss the implication for GBM-PNETs diagnosis/prognosis.

Materials and methods

Materials

All GBM-PNETs were reviewed by JYL as in-house or consultation cases between 2008 and 2011. At least one other neuropathologist also reviewed these cases. Clinical information and pathological findings were obtained by reviewing electronic medical records according to IRB regulation. Specimens were formalin-fixed and paraffin-embedded (FFPE). Four-micrometer-thick sections were prepared for hematoxylin and eosin (H&E) and immunohistochemical staining.

Immunohistochemistry

Immunohistochemistry was performed on an automated immunostainer (Dako Autostainer Link 48). The following antibodies were used: synaptophysin (Ventana, clone SP11, prediluted), GFAP (Dako, clone 6F2, 1:300 dilution), vimentin (Ventana, clone Vim 3B4, prediluted), CD56 (Vector, clone NCAM 1B6, 1:50 dilution), MIB-1 (Ki-67) (Ventana, clone 30-9, prediluted), anti-human IDH1 R132H (Dianova GmbH, Hamburg, Germany, cloneH09, 1:20 dilution), and p53 (Dako clone DO-7, 1:100 dilution). All tissue sections underwent heat-induced antigen retrieval prior to immunostaining.

IDH1 and IDH2 mutation analysis by PCR and pyrosequencing

IDH1 and IDH2 mutation analysis was performed with modification to protocols previously described [4, 25]. Briefly, DNA was isolated from FFPE tissues by using the QIAamp DNA Blood and Tissue Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer’s protocol. PCR was performed in a total volume of 50 μl using 50 ng DNA, 1.5 U GoTaq DNA Polymerase (Promega) and 0.2 μM of the primers to give PCR products of 75 bp spanning the sequence for codon 132 of the IDH1 gene and 102 bp spanning codon 172 of the IDH2 gene. IDH1: forward primer 5’-GCT TGT GAG TGG ATG GGT AAA-3’ and reverse primer 5’-Biotin-TTG CCA ACA TGA CTT ACT TGA TC-3’; IDH2: forward primer 5’-TCC GGG AGC CCA TCA TCT-3’ and
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Table 1. Clinical findings of 10 cases of GBM with PNET-like components

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (yrs)/Sex</th>
<th>MRI Findings</th>
<th>Significant History</th>
<th>Survival</th>
<th>CSF Dissemination</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>44/F</td>
<td>Enhancing nodular lesion (1.7 x 1.3 cm) in right posterior parietal lobe with marked surrounding FLAIR abnormality and mass effect.</td>
<td>Low grade glioma 8 years prior</td>
<td>Alive at 31 months</td>
<td>Yes</td>
</tr>
<tr>
<td>2</td>
<td>76/M</td>
<td>Left temporal, hemorrhagic, prominent peripheral-enhanced lesion (2.8 x 1.8 x 1.6 cm) with mild surrounding FLAIR abnormality; two smaller satellite lesions and focal restricted diffusion on DWI.</td>
<td>None</td>
<td>Dead at 17 months</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>43/F</td>
<td>Heterogeneous mass in right fronto-parietal region (6.8 x 5.5 x 7.2 cm) with solid enhancing component, mass effect, mild surrounding FLAIR abnormality and obvious solid restricted diffusion on DWI.</td>
<td>None</td>
<td>Alive at 15 months</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>82/M</td>
<td>Irregular, ring-enhancing mass (5.9 x 4.0 x 4.0 cm) in right temporal lobe with mild surrounding FLAIR abnormality and focal peripheral restricted diffusion on DWI.</td>
<td>None</td>
<td>Alive at 7 months</td>
<td>No</td>
</tr>
<tr>
<td>5</td>
<td>47/M</td>
<td>Ring-enhancing, cystic mass (4.2 x 3.7 cm) in left temporal lobe with mild surrounding FLAIR abnormality and very focal restricted diffusion on DWI.</td>
<td>None</td>
<td>Alive at 6 months</td>
<td>No</td>
</tr>
<tr>
<td>6</td>
<td>35/M</td>
<td>Right frontal parasagittal, mostly dural-based, heterogeneously enhancing solid and cystic lesion (5.7 x 5.3 x 4.8 cm) with internal hemorrhage, surrounding moderate FLAIR abnormality, mass effect and obvious large restricted diffusion on DWI.</td>
<td>None</td>
<td>Alive at 4 months</td>
<td>Yes</td>
</tr>
<tr>
<td>7</td>
<td>56/M</td>
<td>Ring-enhancing lesion (4.9 x 3.9 x 3.6 cm) in right frontal lobe with marked surrounding FLAIR abnormality and mass effect.</td>
<td>GBM 7 months prior</td>
<td>Dead at 13 months</td>
<td>No</td>
</tr>
<tr>
<td>8</td>
<td>37/M</td>
<td>Diffusely infiltrative, left frontal, temporal and basal ganglia lesion with patchy enhancement, mild mass effect and central restricted diffusion on DWI.</td>
<td>None</td>
<td>Dead at 18 months</td>
<td>No</td>
</tr>
<tr>
<td>9</td>
<td>65/M</td>
<td>Irregular, ring-enhancing lesion (4.6 x 3.6 cm) in right temporal lobe with moderate surrounding FLAIR abnormality and mass effect.</td>
<td>None</td>
<td>Alive at 4 months</td>
<td>No</td>
</tr>
<tr>
<td>10</td>
<td>78/M</td>
<td>Two ring-enhancing lesions in genu of corpus callosum (2.0 x 1.7 cm) and left frontal lobe (3.7 x 1.2 cm) with mild surrounding FLAIR abnormality and peripheral restricted diffusion on DWI.</td>
<td>None</td>
<td>Alive at 2 months</td>
<td>No</td>
</tr>
</tbody>
</table>

DWI=diffusion weighted imaging; FLAIR= fluid attenuated inversion recovery; GBM=glioblastoma multiforme

Results

The clinical information, imaging findings, and pathologic features are summarized in Tables 1 and 2. The median age of the 10 patients was 51.5 years and ranged from 37 to 82 years. The male to female ratio was 4:1. Case 1 had a history of low-grade glioma, which was treated with gross total resection 8 years previously. Case 7 had a history of GBM status post biopsy, radiation and chemotherapy one year prior to the
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The remaining cases were all initial diagnoses. The clinical presentations were non-specific and similar to those from high grade primary brain tumors, such as headache, seizure, nausea and/or vomiting, blurred vision, and motor and/or sensory neurologic defects. Four cases occurred in the temporal lobe, three cases in the frontal lobe, one in the fronto-temporal region, one in the fronto-parietal region and one in the occipital lobe. On MRI, the majority of cases (7 out of 10) showed ring-enhancing lesions; two cases exhibited solid/nodular enhancing lesions; and one case demonstrated patchy enhancement in the tumor. DWI was available for 7 out of 10 cases; there was some restricted diffusion in each case ranging from focal to diffuse (Figure 1). After subtotal resection, 9 out of 10 patients received local radiation therapy and temozolomide. One 82-year old patient was treated with temozolomide only after open biopsy. Dissemination of the tumor through CSF occurred in 2 cases (cases #1 and #6) as demonstrated by MRI. Three out of ten (33%) patients died between 13 and 18 months (median=17 months) from time of initial diagnosis. Seven patients were still alive after 2 to 31 months (median=6 months) following initial diagnosis. Median follow-up was 10 months and ranged from 2 to 31 months.

A GBM component was present in all cases with infiltrating growth pattern, which was composed of fibrillary, gemistocytic, and giant cell astrocytes or a combination of these elements. The

<table>
<thead>
<tr>
<th>Case</th>
<th>Glial Component</th>
<th>PNET Component</th>
<th>IDH</th>
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<tbody>
<tr>
<td></td>
<td>Morphology</td>
<td>CD56</td>
<td>GFAP</td>
</tr>
<tr>
<td>1</td>
<td>Fibrillary astrocytes</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Fibrillary astrocytes</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Astrocytic giant cells and fibrillary astrocytes</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Astrocytic giant cells, gemistocytic and fibrillary astrocytes</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Gemistocytic and fibrillary astrocytes</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Fibrillary astrocytes and rare giant cells</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Mostly, gemistocytic astrocytes</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>8</td>
<td>Mostly, fibrillary astrocytes</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Mostly, fibrillary astrocytes</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>Mostly, fibrillary astrocytes</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

ND=not done; Vim=vimentin; Syn=Synaptophysin; CMB=Classic medulloblastoma; AP/LC=Anaplastic/Large cell medulloblastoma; EA=entrapped astrocytes
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PNET-like component often formed well-demarcated nodules within the glioblastoma background (Figure 2A). In two cases, the PNET-like component formed tiny perivascular nodules (Figure 3A). Tumor cells in the PNET-like component had high nuclear/cytoplasmic ratios, oval, slightly elongated or angulated hyperchromatic nuclei, markedly increased mitotic activity, and obvious apoptotic bodies. Homer-Wright rosettes were noted in all cases. While the PNET-like component resembled classic medulloblastoma in 3 cases, large cell/anaplastic cytologic morphology was seen in the remaining cases (Figure 2B and C).

Case 7 showed classic GBM-PNET histology and therefore, no immunostains were done at initial diagnosis. Moreover, we were unable to access tissue blocks for this case. Tissue blocks were also not available for mutation analysis in case 8. In nine cases with immunohistochemistry results, astrocytic components of tumor showed immunopositivity for GFAP, which was patchy, focal or diffuse (Figure 3C). Gemistocytic and giant astrocytic cells were strongly reactive to GFAP. Synaptophysin was strongly positive in PNET-like foci in all 9 cases (89%) (Figure 2D). Seven out of 9 cases (78%) showed strong and diffuse nuclear staining (70% to 90% nuclei) for p53 in both glial and PNET-like components (Figure 3B). The MIB-1 labeling index was much higher in the PNET-like component (40% to >90%) than that in the glial component (10-40%). The MIB-1 labeling index in the PNET-like component resembling classic medulloblastoma was lower than that in PNET-like component with anaplastic/large cell morphology. Both glial and PNET-like components were strongly immunoreactive for CD56 (Figure 4B, E and F). Vimentin was essentially negative in PNET-like components in 8 out of 9 cases (89%), except as highlighted by rare entrapped astrocytes and blood vessels (Figure 3D, Figure 4A, C and D). All cell types in the glial components of 9 out of 9 cases were strongly and diffusely positive for vimentin. Eight cases were analyzed for IDH1 (R132) and IDH2 (R172) mutations by PCR and pyrosequencing, which showed 2 cases (cases #1 and #3) with R132H IDH1 mutations, and none with mutations in codon 172 of the IDH2 gene. These two cases were stained by R132H mutation-specific IDH1 antibody and were positive for IDH1 in both glial and PNET-like components. Overall, two out of eight (25%) GBM-PNETs carried IDH1 mutations.

Discussion

Primary brain tumors with both glioma and PNET-like components are rare and bear different names in the literature, such as mixed GS and PNET, GS with primitive neuroectodermal differentiation, malignant supratentorial glial-neuronal neoplasms, mixed glioblastoma-cerebral neuroblastoma, or PNET with a GBM component [15, 13, 26, 20, 17, 14]. In a recent large series of 53 patients reported by Perry et al, the term “malignant glioma with PNET-like component (MG-PNET)” is used; the authors suggest that alternate terms such as GBM-PNET, GS-PNET and anaplastic oligodendroglioma (AO)-PNET can be used to indicate specific glial components in specific cases [12].

Perry et al provided evidence supporting a secondary GBM origin for most of their GBM-PNET cases [12]. Our results point toward similar conclusions: our cases had a slightly younger aver-
age of-onset (51.5 years) as compared to those in the study by Perry et al. (54 years) [12], although slightly higher than that typically quoted for secondary GBMs (45 years) [27], and significantly lower than that documented for primary GBMs (62 years) [28, 27]. Also, our cases demonstrated a common strong and diffuse immunopositivity for p53, which is in keeping with a presumptive secondary GBM origin. Moreover, IDH1 mutation, which is frequently associated with secondary GBMs and rarely with primary GBMs [5], was detected in 2 out of 8 patients one of whom had a previous history of low-grade glioma in our study. While IDH1 mutations were found in only 2 cases of the current series, this is still more than one would expect from primary GBMs (7%) [5]. Nevertheless, the overall number of cases analyzed is admittedly small, so these findings will require further evaluation.

The median survival time in the study by Perry et al. was 9.1 months, which is close to that seen for conventional GBMs (9.9 to 14 months) [27, 29]. However, in the current study the median survival time was 17 months for the three patients who succumbed to disease. Moreover, the two patients with IDH1 mutation detected in their tumors were still alive 14 and 31 months after diagnosis. Considering these data, albeit on few cases, it seems plausible to assume a better prognosis for patients diagnosed with GBM-PNET as compared to those with conventional GBMs. However, the overall number of cases is small. The finding requires larger-scale studies for further evaluation.

There are two major hypotheses for the origin of the PNET component in malignant gliomas [12]. The first one is neuroblastic/neuronal metaplasia. The second hypothesis is that clonal expan-
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...tumor stem cells or progenitor cells results in PNET-like nodules. Tumor stem cells/progenitor cells have been discovered in the vascular niches of GBMs, which supports the second hypothesis [30, 31]. Interestingly, in two of our cases (cases 1 and 6), the tiny nodules of PNET-like components were located around blood vessels, which seems to be good histological evidence for stem cell/progenitor cell expansion.

MRI findings for GBM-PNETs are similar to that of conventional GBMs. DWI is used to measure the random motion of water in the tissue. The motion of water decreases as cellularity increases, which is called restricted diffusion. At least focal or even extensive and obvious restricted diffusion was seen in all of the current cases using DWI. Since PNET-like components almost always exhibit hypercellularity, it is not surprising to see such restricted diffusion in these tumors. Therefore, when restricted diffusion is evident in any high-grade glioma, we strongly advocate thorough histological examination to rule-out the presence of PNET-like components, since GBM-PNETs have a higher chance of developing CSF dissemination than conventional GBMs.

Theoretically, astrocytic tumor cells should be positive for GFAP; however, in practice, GFAP positivity can be focal and weak in high grade gliomas, especially in small glioma biopsies. Synaptophysin can give very high background in some tumors and some glial neoplasms can be positive for synaptophysin. Unfortunately, high
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Quality GFAP and synaptophysin immunohistochemical staining is required for optimum GBM-PNET diagnosis, which may be lacking in some immunohistochemical laboratories. For example, if GFAP has insufficient sensitivity to discern the minor glial component inherent in GBM-PNETs, a misdiagnosis of a sPNET will result and the patient will receive unnecessary craniospinal radiation therapy. If synaptophysin gives an aberrantly high background, a regular GBM, partially composed of tumor cells with high nuclear/cytoplasmic ratios, may be misdiagnosed as a malignant glioneuronal tumor or GBM-PNET. In our experience, vimentin strongly and diffusely highlights the astrocytic components and is usually negative in the PNET-like component especially at the center of PNET, which ensures that the minor astrocytic component in the tumor will not be overlooked. CD56 is usually strongly and diffusely positive in both astrocytic and PNET-like components of GBM-PNETs. The CD56 and vimentin combination of immunostain patterns in GBM-PNET makes the diagnosis of this challenging entity much easier. Accordingly, other close mimics can be readily ruled-out. Small cell GBMs are composed of monotonous bland cells with uniform oval nuclei with mild hyperchromasia and minimal cytoplasm, which is strongly and diffusely positive for both CD56 and vimentin. sPNETs are usually positive for CD56 and negative for vimentin and lack a glial component [8].

In summary, restricted diffusion on DWI might

Figure 4. The vimentin and CD56 immunohistochemical stain pattern in glioblastoma with PNET-like components. A. At low magnification, the PNET component on the left is negative for vimentin, while the astrocytic component on the right is strongly positive for vimentin. B. At low magnification, both the PNET component on the left and astrocytic component on the right are strongly immunoreactive for CD56. C and D. At high magnification, the PNET component (C) is negative for vimentin, while the astrocytic component (D) is strongly positive for vimentin. E and F. At high magnification, both the PNET (E) and astrocytic (F) components are strongly immunoreactive for CD56.
indicate PNET-like components in high-grade gliomas. The unique immunostain patterns for vimentin and CD56 make the diagnosis of GBM-PNETs much easier and rule-out other differential diagnoses, such as supratentorial PNET and small cell GBM. The prognosis of GBM-PNET might be better than that of conventional GBMs. IDH1 mutations are present in a minority of GBM-PNETs, which seems to argue against a sole secondary GBM origin for these tumors. Further large-scale studies are necessary to confirm these findings.

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