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ORIGINAL PAPER

# Immunohistochemical Study Concerning the Origin of Neurocytoma—A Case Report

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**Abstract** A 26-year-old woman presented with rapid tumor growth in her left frontal lobe during 9 years of observation. Operative findings revealed a massive tumor connected to gelatinous, transparent membranous tissue (MT), which extended from the paraventricular zone and continued into the lateral ventricle. Histological diagnosis was atypical neurocytoma. Immunohistochemical analyses revealed that the tumor was strongly positive for not only neural markers but also a glial marker, while the MT was positive for a neural marker. The Ki-67/MIB-1 labeling index was 9.1% in the tumor body and 0% in the MT. Musashi 1, a marker of neural stem cells, was strongly positive in both the tumor body and the MT. We speculate that the tumor growth was due to a rapid decline of the Musashi 1-positive cells to glial differentiation. These cells may be candidates for the origin of the tumor.

**Keywords** Extraventricle · Musashi 1 · Neural stem cell · Neurocytoma · Origin · Subependymal

**Abbreviations**

| | |
|---|---|
| CT | computed tomography |
| GFAP | glial fibrillary acidic protein |
| HE | hematoxylin–eosin |
| MRI | magnetic resonance imaging |
| MT | membranous tissue |
| NeuN | neuronal nuclear antigen |
| Syn | synaptophysin |
| TuJ1 | tubulin, βIII isoform |

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## Introduction

It has been speculated that neurocytoma may be derived from the bipotential subependymal plate progenitor cells differentiating to neuronal and glial cells [1–8]. Here we report a case of extraventricular neurocytoma showing the existence of neural stem cells in the paraventricular tissue adherent to the tumor.

## Case Report

When the patient was 17 years old, she was involved in a traffic accident. A head computed tomography (CT) scan demonstrated an incidental lesion in the left frontal lobe adjacent to the left anterior horn of the lateral ventricle. The lesion was 2 cm in diameter and demonstrated moderate enhancment on magnetic resonance imaging (MRI) (Fig. 1A). The patient underwent annual examination until she was 23 years old, and the lesion was found not to have grown at all. When she was 26 years old, MRI revealed growth of the lesion to 6 cm in diameter and it compressed the left lateral ventricle (Fig. 1B). At the time of surgery, the tumor progressed to the left lateral ventricle where a gelatinous, transparent membranous tissue (MT), extending from the tumor, attached to the septum pellucidum (Fig. 1C). The MT occurred in the paraventricular zone. It

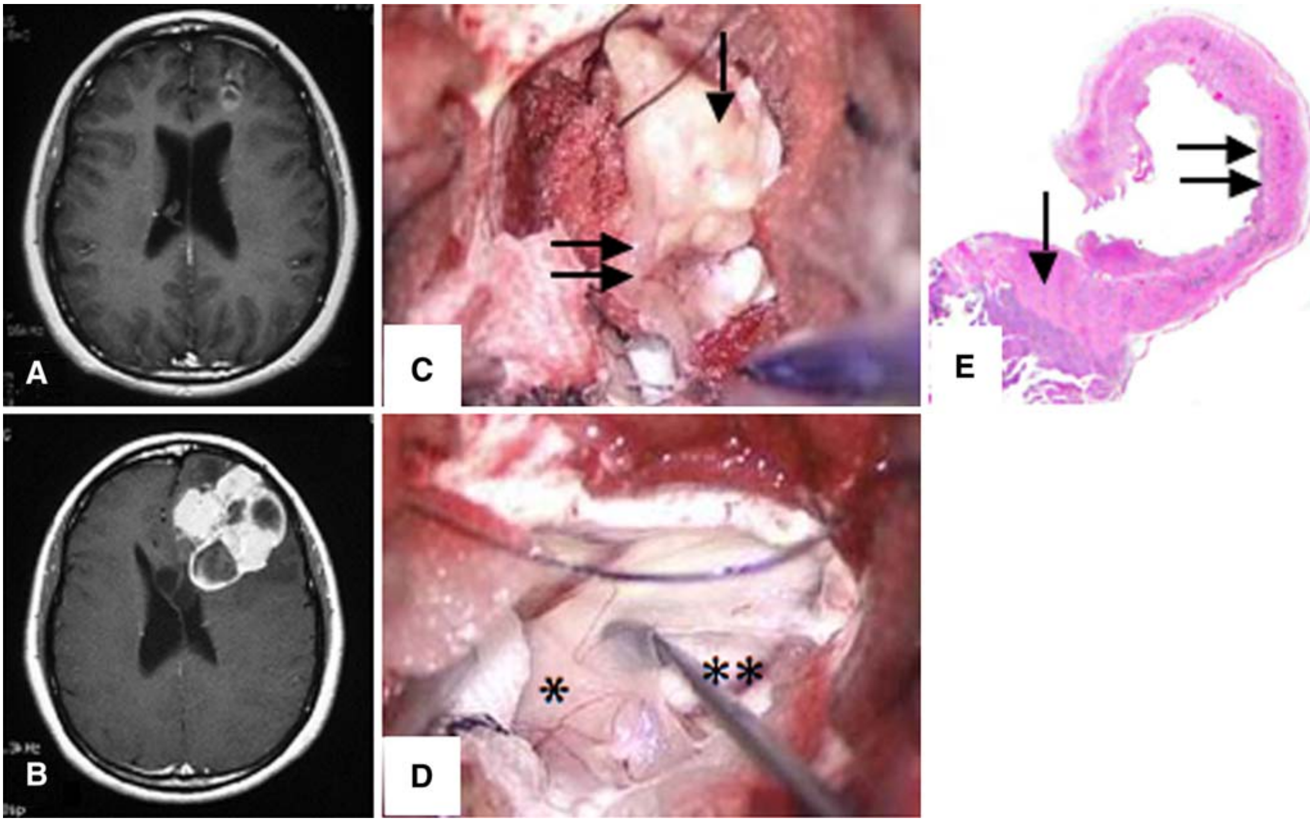


**Fig. 1** **A** Magnetic resonance imaging (MRI) after administration of gadolinium–diethylenetriamine penta-acetic acid (Gd DTPA) performed for the first examination showing a small ring-enhancing lesion in the left frontal lobe. **B** MRI with Gd DTPA on admission 9 years after **A** showing a massively enhanced lesion in the left frontal lobe. **C** Intraoperative photograph showing the left frontal tumor (*arrow*) and the gelatinous, transparent membranous tissue (MT) (*double arrow*) continuing from the tumor into the lateral ventricle attaching to the septum pellucidum. **D** Photograph showing the state of the lateral ventricle after total removal of the tumor body. The end of the MT (*asterisk*) continuing from the tumor was easily torn from the septum pellucidum (*double asterisk*). **E** Combined photomicrograph of hematoxylin and eosin staining showing the whole surgical specimen. The *arrow* indicates the tumor body and double arrow the MT

was moderately easily torn from the septum pellucidum (Fig. 1D). Gross total resection of the tumor was achieved.

## Pathological Examination

Formalin-fixed, paraffin-embedded tissue sections were used. For immunohistochemistry, the following primary antibodies were used: Musashi 1 (Chemicon), glial fibrillary acidic protein (GFAP) (DAKO), synaptophysin (Syn) (Chemicon), tubulin, βIII isoform (TuJ1) (Chemicon), neuronal nuclear antigen (NeuN) (Chemicon), and Ki-67/MIB-1 (DAKO). A non-immunized mouse IgG (DAKO) was used for a negative control. An Envision system (DAKO) was used as a source of secondary antibody and

**Fig. 2** Sections of the tumor body are presented in **A**, **C**, **E**, **F**, and **G**; and those of the membranous tissue (MT) are presented in **B**, **I**, **J**, **K**, and **L**. **D** and **H** show the boundary zone between the tumor body and the MT. **A** Hematoxylin and eosin (*HE*) staining showing cells with uniform small round nuclei and an oligodendroglioma-like halo in the tumor body (original magnification ×200). Cytoplasm-rich cells with small processes, namely astrocytes, were equally observed. Mitotic features were frequent in astrocytic cells (corner, *arrow*, ×400), and prominent endothelial proliferations were often observed. **B** Cells with smaller round nuclei than those in the tumor body and cells with large nuclei, suggesting ganglion cells, were comparatively scattered in the MT section. (×200). **C** GFAP immunostaining disclosing numerous neoplastic astrocytes (*arrow*) and scattered reactive astrocytes (*double arrow*) in the tumor body (×400). **D** Combined photomicrograph of synaptophysin immunostaining showing diffuse strong immunoreactivity in the interstitium of the tumor body (*lower left*) and scattered weakly positive immunoreactivity in the membranous part (*upper right*) (×40). **E** NeuN immunostaining showing a large number of nuclear positive cells in the tumor body (×200). **F** TuJ1 immunostaining showing diffuse positive immunoreactivity in the tumor body (×200). **G** Musashi 1 immunostaining showing a large number of nuclear positive cells (×200). **H** Ki-67/MIB-1 immunostaining of the boundary between the two parts showing strong positive immunoreactivity in the tumor body (*left half*) and absence of immunoreactivity in the membranous part (*right half*) (×200). **I** NeuN immunopositive cells in the MT were less than in the tumor body (×200). **J** Some ganglion like cells (*arrow*) in the boundary zone of the tumor body (*upper left*) showed immunopositive for TuJ1 (×200). **K** Cells with small round nuclei in the MT were negative for GFAP (×400). **L** A large number of nuclei presenting strong positive immunoreactivity for Musashi 1 in the MT (×200)

3,3′-diaminobenzidine was used as chromogen. For electron microscopic examination, tissue sample was immediately fixed in phosphate-buffered glutaraldehyde.

A combined photomicrograph of hematoxylin–eosin (HE) staining demonstrating the whole surgical specimen is presented in Fig. 1E. The HE staining revealed an oligodendroglioma-like halo in the tumor body, while many astrocytes were also observed. Mitotic features and prominent endothelial proliferations were frequently observed. (Fig. 2A). On the other hand, in the MT section, there was a diffuse collection of cells with smaller round nuclei than those in the tumor body. Rare ganglion-like cells were also observed. In the MT section, mitoses and endothelial proliferation were not observed (Fig. 2B).

Immunohistochemistry chiefly demonstrated glio-neural differentiation of the tumor body. A summary of the immunohistochemistry is shown in Table 1. In the tumor body section, GFAP disclosed numerous astrocytes (Fig. 2C). Anti-Syn immunostaining was strongly observed in the interstitium (Fig. 2D). NeuN was widely positive in

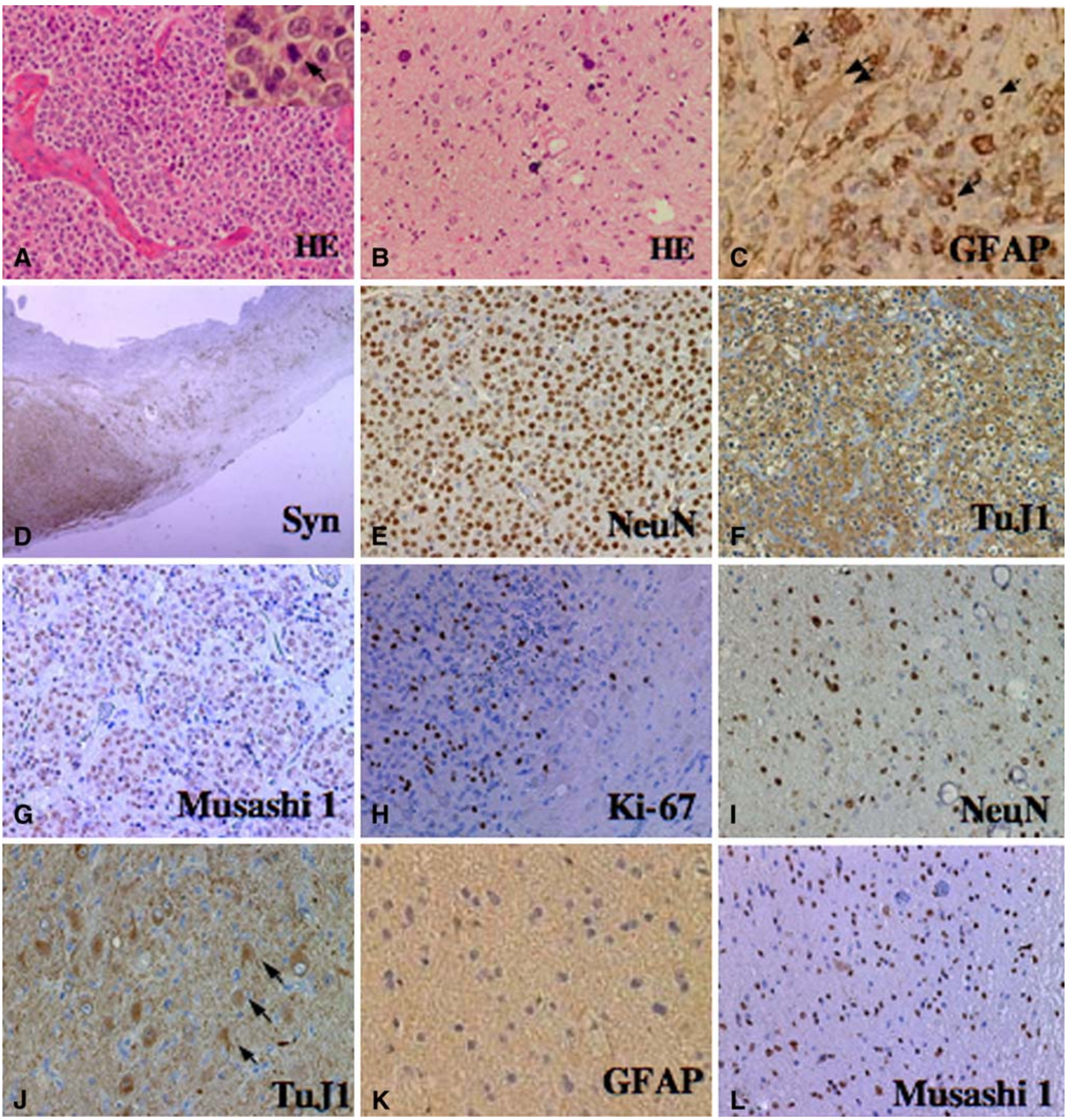

**Table 1** Summary of immunohistochemical findings

| Antibody | Immunoreactivity | |
|---|---|---|
| | Tumor body | Membranous tissue |
| GFAP | + | − |
| Syn | + | +/− |
| TuJ1 | + | +/− |
| NeuN | + | + |
| Musashi 1 | + | + |
| Ki-67/MIB-1 LI (%) | 9.1 | 0 |

+: Positive, +/−: weakly positive, −: negative
*GFAP* glial fibrillary astrocytic protein, *Syn* synaptophysin, *TuJ1* anti-tubulin, βIII isoform, *NeuN* neuronal nuclear antigen, *LI* labeling index

the nuclei of the tumor cells (Fig. 2E). TuJ1 was also widely positive in the cytoplasm of the tumor cells (Fig. 2F). Thus, the tumor body included neoplastic tumor cells that were positive for neuronal and glial markers. Musashi 1 was diffusely positive in the nuclei of the tumor cells (Fig. 2G). The Ki-67/MIB-1 labeling index was 9.1% (Fig. 2H).

On the other hand, in the MT section, there were scattered synaptophysin-positive cells (Fig. 2D). The number of cells positive for NeuN was much less than in the tumor body (Fig. 2I). TuJ1 immunoreactivity was only observed in a small number of neuronal cells (Fig. 2J). GFAP was negative in the cells with small round nuclei (Fig. 2K). Strong immunoreactivity for Musashi 1 was observed in many small nuclear cells (Fig. 2L). Immunostaining for Ki-67/MIB-1 was negative (Fig. 2H). An electron micrograph showed some incomplete synapse-like structures, including a dense core vesicle and some Golgi apparatuses. The above findings led us to diagnose this case as an extraventricular atypical neurocytoma.

## Discussion

Some central neurocytomas have been proposed to arise from a subependymal plate progenitor cell [3, 5, 6], however, no reports have demonstrated the existence of neural stem cells in the subependymal zone as candidates for the origin of neurocytoma. It was difficult to seek the tumor origin in the lateral ventricle because no obvious abnormal signal was detected in the primary images in this case. The MT was considered to be predominantly in the paraventricular area.

Musashi 1 is an excellent marker for neural progenitor cells in the normal human brain [9]. Musashi 1-positive cells exist in the periventricular zone [9, 10]. Uchida et al. [11] demonstrated that an infant's brain tumor originated from neural stem cells located in the subventricular zone. We regarded the Musashi 1 positive cells in our case as neural stem cells that were possible candidates for tumor origin.

The Musashi 1 is reported to play a role in differentiation for neural and glial development [9, 10]. In our present case, the tumor cells retained the potential of glial and neural differentiation. There is a good likelihood that the differentiation of neural stem cells is regulated by Musashi 1 protein.

Neurons have been reported to express TuJ1 early in differentiation [12, 13] and NeuN after maturation [14]. Syn has been known as a marker of the synapse. It was inferred from the results of immunohistochemistry that the MT would demonstrate neural differentiation before maturation; on the other hand, the tumor body would include the cells in some stages of neural differentiation. Accordingly, immature neural structures might be observed in the electron micrograph. It was considered that the MT would be in the pre-tumorogenic stage.

It is reasonable to consider that most Ki-67/MIB-1 positive cells in the tumor body were included in the astrocytic component because a large number of GFAP positive cells were observed only in the tumor body. We speculate that the Musashi 1 positive cells in the tumor might have suddenly and rapidly differentiated into glial cells, resulting in tumor growth for a short period.

In conclusion, we present a case of extraventricular atypical neurocytoma with paraventricular tissue that included Musashi 1 positive cells, which are considered to be possible candidates for tumor origin.

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