

## Evaluation of CD133 expression in brain tumors using tissue array

Keiji Shimizu, Ayano Kumasawa, Shinichi Toyonaga, Takanori Masahira, Hiromochi Nakabayashi, Kaechang Park, Toshio Yawata  
 Department Neurosurgery, Kochi Medical School, Kochi Japan

### Abstract

CD133/prominin is a plasma membrane marker found in several types of somatic stem cells. Various tumor cell lines contain a distinct population of CD133 positive cells and these cells are called cancer stem cells. Because the population possesses the capability for the high tumorigenicity and clonogenicity, it represents an important target for anticancer therapies. Immunohistochemical study for CD133 in brain tumor tissues is still elucidative, because of small number of case and ambiguous staining in previous studies. Therefore, we improved the immunohistochemical staining and analyzed in 171 cases of brain tumor tissues, including 107 astrocytomas, 49 glioblastomas, 11 medulloblastomas and 4 ependymomas tissues using tissue array, for CD133 expression. The expression of CD133 was detected in 1 of 19 grade 1 (5.2%), 7 of 67 grade 2 (10.4%), 10 of 21 grade 3 (47.6%) astrocytomas, 35 of 49 glioblastomas (71.4%), 1 of 11 medulloblastomas (9.1%) and 4 of 4 ependymomas (100.0%). Average percentage of CD133-positive cells was 0.1% in grade 1, 1.9% in grade 2, 6.7% in grade 3 astrocytomas, 18.5% in glioblastomas, 0.2% in medulloblastomas and 38.7% in ependymomas, demonstrating a statistically significant association with histological grade. This result indicates that CD133 is shown to be a strong diagnostic marker for glioma malignancy except for ependymomas and medulloblastomas and the existence of cancer stem cells affects pathologic grade of astrocytomas.

### Introduction

Glioblastoma (GBMs) remain the most lethal of primary brain tumors because the median survival time is less than 12 months and the prognosis have not changed during the past 20 years in spite of surgical removal, radiation and chemotherapy. GBMs frequently recur or progress after multimodality therapy as focal masses, suggesting that subpopulation of tumor cells is responsible for regrowth. Recently, several groups identified cancer stem cells (CSCs) in brain tumors. They used CD133, a marker of subset of neural stem cells, to isolate cancer stem cells and showed the capacity for unlimited self-renewal, as well as the ability to initiate and drive tumor progression in animal models. These findings suggest that the population of CSCs could affect to tumor grade and prognosis. The aim of this study was to explore immunohistochemically the correlations between CD133 expression and grade of malignancy in human brain tumors.

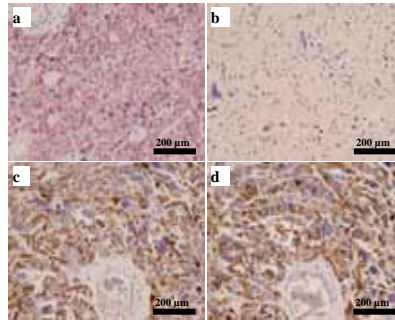
### Materials & Methods

#### Tissue samples and immunohistochemistry.

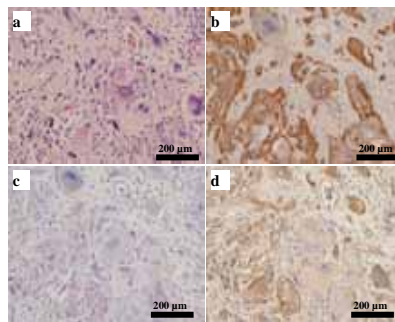
Surgical glioblastoma specimens were obtained from 60-years old female patient. Synthetic peptide KDHVYGIHNPVMTSPSQH were used in immuno-blocking experiments (25 µg/ml). Tissue microarrays containing normal and brain tumor tissues were obtained from BioMax Inc. (Rockville, MD). The data available for the samples included the age, gender, and pathological diagnosis of the patients. Immunohistochemistry. Tissue sections were first deparaffinized with xylene (5 min, three times), hydrated gradually through a series of graded ethanol (100%, 90%, 80%, 70%) and air-dried. To quench the endogenous peroxidase activity, the sections were incubated with methanol containing 0.6% H<sub>2</sub>O<sub>2</sub> for 20 min. After washes with phosphate-buffered saline (PBS), the sections were pretreated for antigen retrieval in a microwave oven for approximately 20 min in citrate buffer and cooled for 25 min at room temperature. After washes with PBS, nonspecific binding was blocked with 1% normal horse serum for 40 min at room temperature. The sections were then incubated with the rabbit anti-CD133 antibody (Abcam, 1:100 dilution) in a humidified chamber overnight at 4 °C. On the next day, the sections were rinsed three times in PBS for 3 min, each, and incubated with biotinylated goat anti-rabbit IgG (Cedarlane, Burlington, NC). CD133 was developed with Vector VIP peroxidase substrate (Vector Laboratories) to give a purple reaction product.

#### Statistical analysis

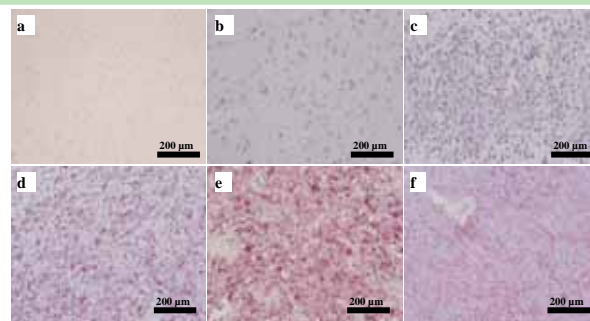
The ordinal data were analyzed according to the Kruskal-Wallis test with support of the standard software "NoPAS". Correlations between normal brain and GBMs with  $P < 0.05$  were considered significant.



Characterization of antibodies using blocking peptides used in raising anti-CD133 antibody. Immunohistochemical analysis of paraffin-embedded human glioblastoma were performed using rabbit anti-CD133 antibody (a). The specimen were also preincubated with blocking peptides used in raising anti-CD133 antibody (b). The expression of nestin was observed after no (c) or treatment with the blocking peptide (d). The anti-CD133 antibody specifically reacted with stem cells dependent on peptide sequence. Addition of the blocking peptide inhibited the stainability of anti-CD133 antibody, but not anti-Nestin antibody.



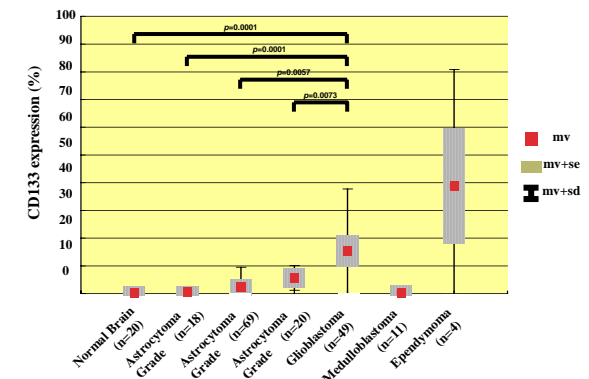
Coincidence of CD133 and Nestin immunostaining in glioblastoma specimens. Serial sections from patient with GBM were stained with H&E(a) and immunohistochemical reagents for expression of GFAP(b), CD133(c) and Nestin(d). CD133 positive cells also express nestin, suggesting that anti-CD133 antibody is useful for the detection of stem or immature cells.



Examples of CD133 expression levels observed in brain tumors by immunohistochemistry. (a) Normal tissue, (b) Astrocytoma grade I, (c) grade II, (d) grade III, (e) glioblastoma and (f) ependymoma.

Detection of CD133 expression in brain tumors using tissue array. One hundred ninety-one cases including 20 normal brain, 107 astrocytomas, 49 glioblastomas, 11 medulloblastomas and 4 ependymomas were stained with anti-CD133 antibody.

	CD133 positive cells					Total (%)
	0%	<10 %	<30 %	<50 %	>50 %	
Normal brain (n=20)	20					0
Astrocytoma grade I (n=19)	18	1				5.2
Astrocytoma grade II (n=67)	60	4	1	2		10.4
Astrocytoma grade III (n=21)	11	7	3			47.6
GBM (n=49)	14	11	13	6	5	71.4
Medulloblastoma (n=11)	10	1				9.1
Ependymoma (n=4)		2			2	100



Correlation between WHO grade and expression of CD133 in brain tumors (mv mean value, sd standard deviation, se standard error)

### Conclusion

Rabbit anti-CD133 used in this study is specifically reactive with stem or immature cells expressing nestin in paraffin-embedded tissues.

The expression of CD133 is strongly correlated with grade of astrocytic tumors. The existence of cancer stem cells may affect pathologic grade of astrocytomas.

Minor population of glioblastoma specimens is not expressing CD133, suggesting that CD133 may be not always stem marker, and that cancer stem cells do not exist in some tumor.

CD133 is expressed in all ependymoma tissues examined in this study, leading the expression may be dependent on tumor origin.