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Expression of CD133 and Extracellular Matrix Molecules in Malignant Brain Tumors

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ABSTRACT

***Background*:** *CD133 could be characterized as a "stem-like" cell subpopulation and an invasive tumor phenotype. The objectives of this study were to investigate the relationship of CD133 and other remodeling factors such as matrix metalloproteinases (MMP) in the brain tumors.* ***Methods*:** *Tumors from 13 patients with brain tumors (8 lung cancer metastasis, 3 breast cancer metastasis, 2 gliomas) were studied to investigate the expression-patterns of CD133, EGFR, MT1-MMP, and MMP7 using the immunostaining and RT-PCR analysis.* ***Results*:** *EGFR immunostaining was detected in 75% (6/8) and 67% (1/3) of brain metastasis from lung adenocarcinoma and breast cancer, respectively. MT1-MMP immunostaining was also detected in 73% (8/11) of these brain metastasis. CD133 was not detected in these 13 patients. EGFR immunostaining was detected in 75% (6/8) and 67% (1/3) of brain metastasis from lung adenocarcinoma and breast cancer, respectively. MT1-MMP immunostaining was also detected in 73% (8/11) of these brain metastasis. CD133 was not detected in these 13 patients.* ***Conclusions*:** *The expression of CD133 indicates a marker for brain tumor initiating.*

***Keywords*:** *CD133, EGFR, MT1-MMP, MMP7, Brain Tumors*

1. Introduction

A small population of cancer stem cells (CSCs) within neoplasm is supposed to be responsible for long-term tumor proliferation [1]. Some groups have also subsequently showed that CSCs exist in brain tumor, [2], and CD133 could be characterized as a marker of CSCs subpopulation and an invasive tumor phenotype [3,4].

The malignant brain tumors contain CSCs are also thought to show higher NOTCH activity [5]. The NOTCH pathway may be also related to many remodeling factors such as matrix metalloproteinases (MMP) in the brain tumors. It is reported that the NOTCH pathway represents a possible target in stem-like malignant brain tumor cells [6]. Furthermore, it has recently been reported that common chemotherapeutic drugs, as well as traditional radiation therapy, predominantly targeted the CD133-negative population [7,8]. The NOTCH pathway represents a possible target in stem-like malignant brain tumor cells [9,10]. In this study, we investigate the relationship of CD133 and other remodeling factors such as MMPs concerning with the NOTCH pathway in the malignant brain tumors [11,12].

2. Patients and Methods

Tumors from 15 patients with malinant brain tumors (**Table 1**) were studied to investigate the expression-patterns of CD133, EGFR, MT1-MMP, MMP7, and CD44 using the immunostaining and RT-PCR analysis.

3. Immunostaining

All these drugs are reported to be unable to penetrate the blood-brain-barrier. Sections of paraffin-embedded tissues were studied by the immunohistochemistry(IHC) using monoclonal antibodies to CD133, EGFR, MT1-MMP, MMP7, and CD44 (Oncogene Research Labs). Using the methods described previously [13], 8-micro mm sections were treated using the avidin-biotin-peroxidase complex method (Vectastatin Elite ABC kit; Vector Labs). Endogenous peroxidase activity was blocked with 3% H_2O_2 in methanol for 30 min. Sections were blocked with 20% horse serum for 1 h. Primary antibody at 1:100 dilution was applied overnight at 40˚C. Secondary biotinylated antibody at 1:200 dilution was applied for 1 h to visualize the bound antibody. ABC reaction was performed for 1 h at room temperature. The peroxi-

Table 1. Characteristics of patients.

Tumor type	Age (yr) Average TTD[a] from Met[b] (mo)	Previous chemotherapy[c]	
		No	Yes
Malignant glioma (n = 2)	48-68	2	0
Lung carcinoma adeno (n = 8)	47-73	9.3	35
squamous (n = 2)	65-77	7.6	11
Breast carcinoma (n = 3)	38-62	14.2	30

[a]TTD, time to death; [b]Metbrain metastasis; [c]chemotherapy, seven of the 15 patients had been treated for the primary lesions with anticancer drugs.

dase activity was developed by incubation in 0.05% 3,3-diaminobenzidine for 3 min. Control sections were obtained from the surrounding brain tissues where no cancer cells were detected.

4. Reverse Transcription/Polymerase Reaction (RT-PCR)

The obtained brain tumor tissues were frozen in liquid nitrogen and stored at –80˚C. Total RNA was prepared using the RNase Mini Kit according to the manufacture directions(Qiagen, Chatsworth, CA). The RNA samples were reverse-transcribed using Superscript reverse transcriptase (Life Technologies Inc., Gaithersburg, MD.), random hexamers, and dNTPs. cDNA synthesized were amplified by PCR(30 cycles) with Taq DNA polymerase (Qiagen, Chatsworth, CA) in the presence of both dNTP and an appropriate pair of primers. The following sense and anti sense primer was used:

CD133 forward primer 5'-CAG AGT ACA ACG CCA AAC CA-3',

CD133 reverse primer 5'-AAA TCA CGA TGA GGG TCA GC-3'.

EGFR sense; 5-AGCGGATAACAATTTCACACAGG-3

anti-sense; 5-GTCGTCTTTCCAGACGTTAGT-3

MT1-MMP sense; 5-ACAGTCTGCGGAACGGAGC AG-3

anti-sense; 5-GTCAATTGTGTTTCTGTCCAC-3

MMP7 sense; 5-GTGGTCACCTACAGGATCGTA-3

anti-sense; 5-CTGAAGTTTCTATTTCTTTTTGA-3

CD44 sense; 5-GTACGTCTTCAAATACCA-3

anti-sense; 5-GTGGTTGAAATGGTGC-3

β-actin forward primer 5'-GTC TTC CCC TCC ATC GTG-3',

β-actin reverse primer 5'-AGG TGT GGT GCC AGA TTT TC-3',

Results were expressed as band intensity in each lane relative to GAPDH and compared statistically using Student's t test.

5. Statistical Analysis

The immunohistochemical and PCR activities measured in this study were analyses using the Student t-test. A probability value less than 0.05 was considered statistically significant.

6. Results

6.1. Immunohistochemistry

EGFR immunostaining was detected in 75% (6/8) and 67% (1/3) of brain metastasis from lung adenocarcinoma and breast cancer, respectively. MT1-MMP immunostaining was also detected in 73% (8/11) of these brain metastasis. CD133 was not detected in these 13 patients. EGFR immunostaining was detected in 75% (6/8) and 67% (1/3) of brain metastasis from lung adenocarcinoma and breast cancer, respectively (**Figure 1**). MT1-MMP immunostaining was also detected in 73% (8/11) of these brain metastasis. CD133 was not detected in these 13 patients (**Table 2**).

6.2. Identification of Gene Expression

Figure 2 shows the relative gene expression levels of brain metastasis from lung cancer for EGFR, MT1-MMP, MMP7, and CD44. **Table 3** summarizes the RT-PCR results. EGFR mRNA was detected in 83.3% (25/30) and 70% (7/10) of brain metastasis from lung cancer and breast cancer, respectively. MT1-MMP mRNA was detected in 73.3% (22/30) specimens of brain metastasis from lung cancer, and only 13.3% (4/30) specimens of these patients expressed MMP7 mRNA. MT1-MMP mRNA was also detected in 50% (5/10) of brain metastasis from breast cancer. As for the correlation between EGFR and other gene expression, gene expression of

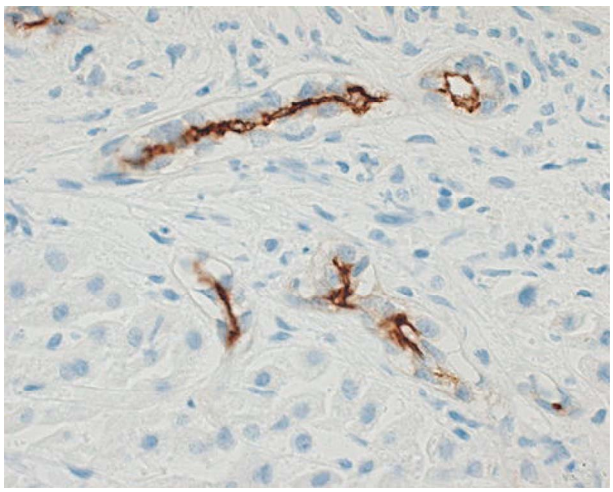

Figure 1. Immunohistochemical analysis.

Table 2. Immunostaining of brain metastasis.

Stain	lung cancer met[a] (n = 10)		breast cancer met (n = 3)		malig.glioma (n = 2)	
	(+)	(–)	(+)	(–)	(+)	(–)
CD133	(1/10)	(9/10)	(0/3)	(0/3)	(1/2)	(1/2)
EGFR	(6/10)	(4/10)	(1/3)	(2/3)	(2/2)	(0/2)
MT1-MMP	(7/10)	(2/10)	(1/3)	(1/3)	(1/2)	(1/2)
MMP7	(1/10)	(7/10)	(1/3)	(1/3)	(0/2)	(2/2)
CD44	(0/10)	(10/10)	ND[b]	ND	(0/2)	(1/2)

[a]met; brain metastasis from lung adenocarcinoma, [b]ND; not done.

Table 3. Summary of detection of CD133, EGFR, MT1-MMP, MMP7, and CD44 mRNA expression in brain tumors by RT-PCR technology.

mRNA	lung cancer met.[a] positive No.of meta[c]/total evaluated	breast cancer met. positive No.of meta[d]/total evaluated	Malig.glioma positive No/total evaluated
CD133	2/10 (20%)	0/3 (0%)	1/2 (50%)
EGFR	8/10 (80%)	1/3 (33%)	1/2 (50%)
MT1-MMP	7/10 (70%)	1/3 (33%)	1/2 (50%)
MMP7	1/10 (10%)	1/3 (33%)/5	0/2 (0%)
CD44	0/10	ND[b]	0/2 (0%)

[a]met; brain metastasis, [b]ND; not done. [c]meta; brain met. from lung adenocarcinoma, [d]met; brain meta. from lung squamous cell cancer.

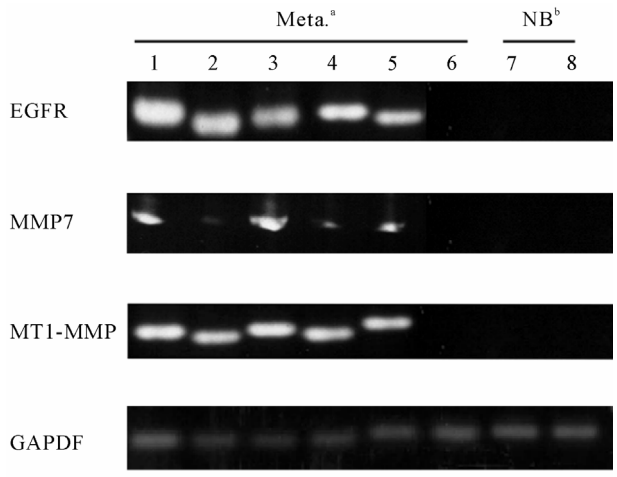

Figure 2. The relative gene expression levels of brain metastasis.

MT1-MMP was relatively correlated with that of EGFR. That is, 63.3% (19/30) of brain metastasis from lung cancer expressed both EGFR and MT1-MMP mRNA. However, we could not find any statistical significances in this correlation. No difference in the expression of MMP7 and CD44 was also observed between brain metastasis and normal brain tissue. Furthermore, we could not find any differences of these gene expression in the relationship with the age, sex, and tumor sites (data not shown). There was also no difference in these gene expression between adenocarcinoma and squamous cell carcinoma. Only 4 cases expressed neither detectable levels of EGFR nor MT1-MMP mRNA by RT-PCR. The GAPDH in these cases, however, was detected with similar levels in all cases. These tendency was also observed in brain metastasis from breast cancer. **Figure 3** shows the expression of CD133mRNA in patients with lung cancer.

7. Discussions

A number of groups have showed that CSCs exist in brain tumors, and CD133 could be a marker of CSCs subpopulation [14]. These CSCs and NOTCH pathway are thought to be important in the proliferation of malignant brain tumors [15].

In the previous study, we have demonstrate that EGFR and MT1-MMP were immunostained predominantly in brain metastasis [13]. The high expression of EGFR and MT1-MMP indicates that an extracellular matrix remodeling may be playing an important role in brain metastasis [16]. In this current study, EGFR immunostaining was detected in 75% (6/8) and 67% (1/3) of brain metastasis from lung adenocarcinoma and breast cancer, respectively. MT1-MMP immunostaining was also detected in 73% (8/11) of these brain metastasis. CD133 was not detected in these 13 patients. Although most cases showed the poor expression of CD133, two patients with malignant brain tumors showed the positive staining of CD133. In these cases, EGFR and MT1-MMP were strongly stained.

Therefore, the potential therapeutic implications of targeting of stem-like cells via NOTCH inhibition extend beyond neuro-oncology, as this signaling pathway has been shown to play a role in CSCs not only in neural tumors, but also in the brain metastasis fro lung and breast cancer [17-19]. More studies will be needed to determine whether pharmacological NOTCH blockade, either alone or in conjunction with other therapies, will be effective in improving the survival of patients with GBM and other malignant tumors.

8. Conclusions

The expression of CD133 indicates a marker for brain tumor initiating.

More studies will be needed to determine whether pharmacological NOTCH blockade, either alone or in

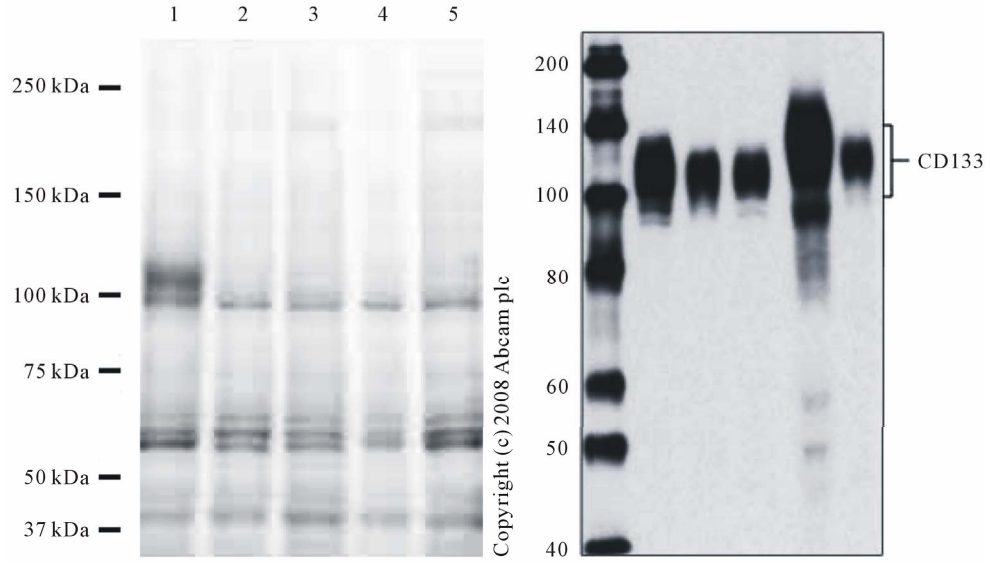

Figure 3. Expression of CD133 mRNA（lung cancer metastasis). Repeated SDS -PAGE analysis of the cerebrospinal fluid and the urine of the 54 years old patients with malignant glioma. Lane 1-2: urinary sample; lane: 3 CSF sample; lane 4: CSF sample at end stage.

conjunction with other therapies, will be effective in improving the survival of patients with GBM and other malignant tumors.

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