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RESEARCH

Expression of Cytokeratins in Glioblastoma Multiforme

Tadashi Terada

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Abstract Little is known about the cytokeratin (CK) expressions in glioblastoma multiforme (GBM). The aim is to explore the CK expression in GM using immunohistochemistry (IHC). IHC study in 30 cases (median=68 years, SE=12.6) of GBM in brain. CK expression using AE1/3 antidody was seen in 29/30 (97 %) cases. There were no expressions of CK34BE12, CK5, CK6, CK7, CK8, CK14, CK 18, CK19, and CK20. Expression of p53 and glial fibrillary acidic protein (GFAP) were recognized in all 30 cases (100 %). All cases showed Ki-67 antigen labeling, index of which ranged from 6 to 43 % (m±SD=24+16). The IHC using CKAE1/3 in GBM very frequently shows positive reaction. The expression may cause difficulty in pathologic diagnosis in GBM, particularly in discrimination between GBM and metastatic carcinoma. The CK positivity in GM may be due to CK molecules other than CK34BE12, CK5, CK6, CK7, CK8, CK14, CK18, CK19 and CK20. GBM frequently expression p53 and high Ki-67 labeling.

Keywords Glioblastoma multiforme · CK · Immunohistochemistry

Introduction

Glioblastoma multiforme (GBM) is a relatively common malignant tumor of glial cells. It is histologically characterized by atypical glial cells, mitosis, necrosis, and vascular endothelial proliferation. Of the above four criteria, the presence of two elements implies anaplastic astrocytoma (WHO Grade 3), and the presence of three or more elements shows GM (WHO Grade 3).

Of the five intermediate filaments (IF), glial fibrillary acidic protein (GFAP) is localized in glial cells and their tumors including GBM, and cytokeratins in the epithelial cells. However, the myth of localization of IF collapsed because it became clear that some carcinomas, for example, express vimentin in addition to CK, and that some sarcomas show CK as well as vimentin. This is the case with brain tumors. In GBM, there has been only a few reports of expression of CK in GM [1–6]. However, the results of CK expression have been conflicting and there are no comprehensive reports on expressions of CK subtypes [1–6].

Brain tumors are usually diagnosed in frozen rapid sections and post-frozen formalin-fixed and paraffin embedded sections, because the brains tumors cannot be examined by biopsy. The author has usually confronted GBM case difficult to diagnose and differentially diagnose, thus prompting the author to investigate CK in human GBM.

Materials and Methods

The author collected 30 cases of GBM from the computer data base of the author's laboratory. The materials had been fixed in 10 % formalin and embedded in paraffin. The median age of the 30 cases was 68 years with SD of 12.6 years, and male to female ratio was 17:13. The paraffin blocks were stained with hematoxylin and eosin and with the use of Envision method [7] immunostainins. The following antibodies were examined: CKAE1/3 (cocktail, Ventana, 1:100), anti-high-molecular-weight keratins (CK34BE12)(polyclonal, Dako, 1:100), anti-CK5 (clone 6D5/16 B4, Dako, 1:150), anti-CK6 (clone 6D5/16 B4, Dako, 1:150), anti-CK7 (clone OV-TL, Dako Corp, dilution=1:200), anti-CK8 (Clone 35bH11, Dako, 1:150), anti-CK14 (clone NCL-LCK14, Novocastra,

T. Terada (✉)
Department of Pathology, Shizuoka City Shimizu Hospital, Miyakami 1231, Shimizu-Ku, Shizuoka 424-8636, Japan
e-mail: piyo0111jp@yahoo.co.jp

1:200), anti-CK18 (clone DC10, Dako, 1:200), anti-CK19 (clone BA17, Dako, 1:100), anti-CK20 (clone Ks20.8, Dako, 1:200), anti-GFAP (polyclonal, Dako, 1:500), anti-p53 (clone DO7, Dako, 1:300) and anti-Ki67 (MIB1, Dako 1:100). Microwave pretreatment was performed by each immunohistochemical run. Positive and negative appropriate control tissues were stained in each run.

Results

Histologically, GBM showed proliferation of atypical spindle cells (Fig. 1a) with mitotic activity, vascular endothelial proliferation, or necrosis. Pseudopalisadings were noted in some cases.

Immunohistochemically, CKAE1/3 were recognized in 29/30 cases (9 %) (Fig. 1b). CK34BE12, CK5, CK6, CK7, CK8, CK14, CK18, CK19 and CK20 were negative in all cases. GFAP was positive in all cases (Fig. 1c). All cases of GBM expressed p53 (Fig. 1d) and high Ki-67 (Fig. 1e), labeling index of which ranged from 6 to 43 % (m±SD=24+16 %).

Discussion

CK belongs to IF protein family, and is a powerful useful tool in diagnostic pathology. At present, more than 20 different CKs have been identified, of which CK 8, 18, and 19 are the most abundant in simple epithelial cells. CK provides useful markers for epithelial malignancies. CK34BE12, CK5, CK6 belong to high-molecular weight CK which are expressed in squamous epithelium and mesothelium. CK14 is usually expressed of myoepithelial cells. CK20 is expressed in some epithelium such as colonic epithelium, and it is thought that CK7/CK20 expression can be of potential use in determining cell origin in malignancies.

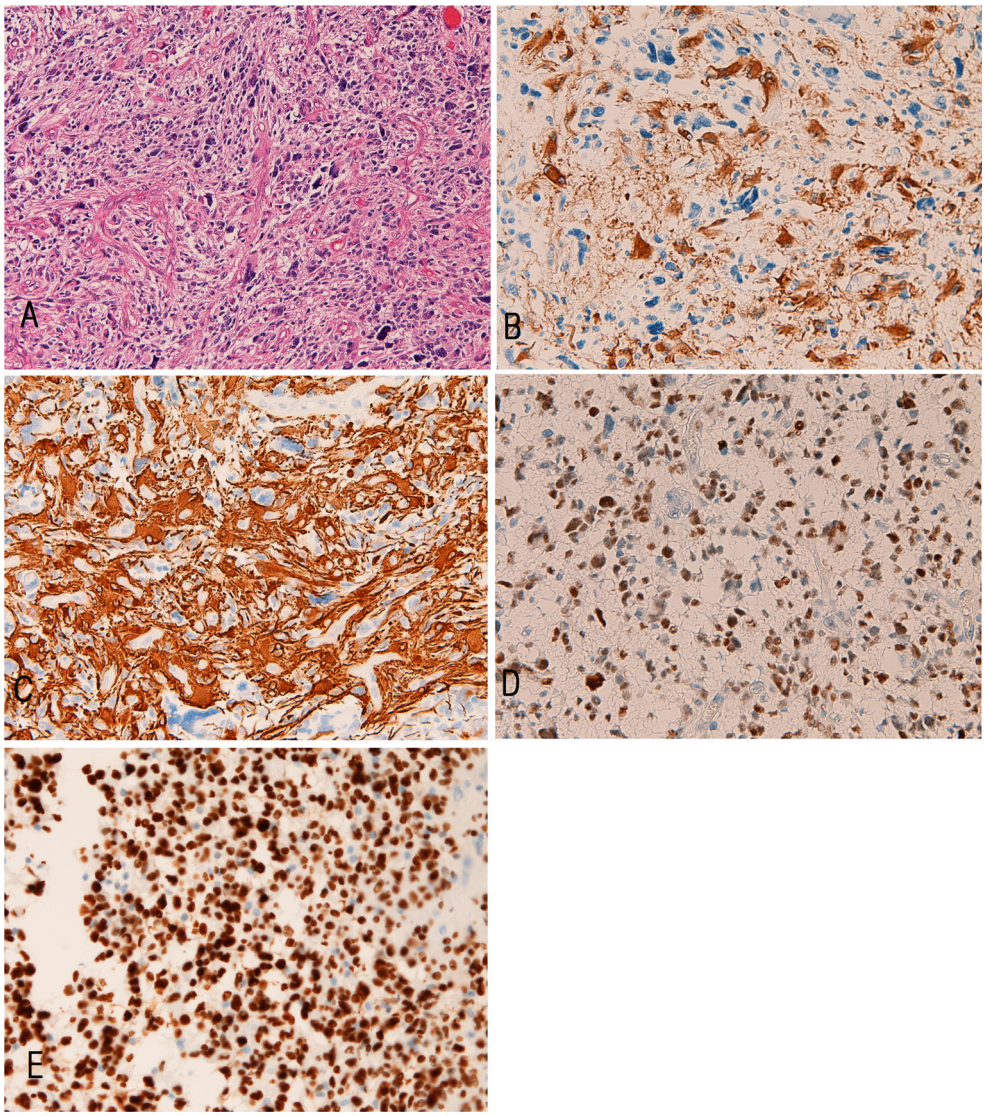

Fig. 1 Histological and Immunohistochemical features of glioblastoma. (**a**): Typical histological features of glioblastoma. HE, x100. (**b**–**e**): Immunohistochemical features. Positive reaction of cytokeratin AE1/3 (**b**), glial fibrillary acidic protein (**c**), p53 (**d**) and Ki67 (**e**). B-E, x200

In the present study, the author investigated expressions of CK detected by the antibody CKAE1/3, in addition to CK34BE12, CK5, CK6, CK7, CK8, CK14, CK18, CK19, and CK20. In the present cases of GBM, the antibody of CKAE1/3 labeled GB cells in 29/30 (97 %) cases, indicating that GBM cells contains certain kind of CK molecules. In the present study. In the present study, no expression (0/30 cases) were seen in immunostainings of CKCK34BE12, CK5, CK6, CK7, CK8, CK14, CK18, CK19, and CK20, suggesting that these subtypes of CK are not expressed in GBM. GFAP was expressed in 30/30 (100 %) of GBM in the present study. In diagnosis of GBM, it is very important to differentiate GBM from metastatic carcinoma, in particular metastatic poorly differentiated and undifferentiated carcinomas. In general diagnostic pathology, immunostainings of IF (CK, vimentin, and GFAP) were performed. The most usual CK immunostainings are CKAE1/3 and CKCAM5.2. The present study suggests that CKAE1/3 positivity does not exclude the diagnosis of GBM. A combination of GFAP and CKAE1/3 is necessary in determination the diagnosis GB. The present study did not employed CKCAM5.2, but CKCAM5.2 may be negative because the present GBM did not expressed CK8 and CK18; CAM5.2 detect CK8 and CK18.

A comparative discussion of previous studies is needed. There have been only six reports in the expression of CK in GBM [1–6]. Cosgrove et al. [1, 3] showed that 10/12 (83 %) GBM showed positive reaction with CKAE1/3. Of the AE1/AE3-positive cases, 58 % reacted with CK34BE12. None of the cases was reactive with CK34BH11. Ng and Lo [2] showed that 7/12 cases (58 %) of GM and 5/8 cases of anaplastic astrocytomas were positive for CKAE1/3. Hirato et al. [4] [showed positive expression of epidermal CK in 20/24 (83 %) gliomas. Oh and Prayson [5] showed that 32/33 (95.7 %) GBM stained positive for cytokeratins AE1/3, and 1/33 (4.3 %) cases showed focal immunoreactivity of CKCAM 5.2, CK7 and CK20. Goswami et al. [6] showed that 7/32 (22 %) astrocytic neoplasms showed focal immunoreactivity with pancytokeratin. The results of the present study somewhat resemble previous study, but expression of CKAE1/3 is much higher in the present study. In addition, the present study showed negative expression of CK34BE12, CK5, CK6, CK7, CK8, CK14, CK18, CK19, and CK20.

The types of CK in GBM are the next step. CK cocktail, CKAE1/3 detects CK1, 2, 3, 4, 5, 6, 7, 8, 10, 14, 15, 16 and 19. CK cocktail CK34BE12 reacts to CK1, 5, 10, 14. The human CK consists of 20 different CK molecules (CK1 to CK20) and includes consisted of two types, the acidic type 1 CK and the basic or neutral type 2 CK. Acidic cytokeratins include CK9, CK10, CK12, CK13, CK14, CK16, CK17, CK18, CK19 and CK20. Basic or neutral cytokeratins include CK1, CK2, CK3, CK4, CK5, CK6, CK7, and CK8. CK is classified into low- versus high-molecular weight based on their molecular weight. The speculation of the subtypes in GBM is simple: CKAE1/3 minis other CK examined. From these observations, it is suggested that the CK of GBM is of one or more of CK 2, 3, 4, 15, 16.

The present study showed consistent expression of p53 and high Ki-67 labling in GBM. These data suggest that p53 mutations and enhanced cell proliferation in GBM. The suspected p53 mutation in the present study is supported by many molecular analyses of p53 gene [8]. The high Ki67 labeling is compatible with previous reports [9].

Conflict of Interest The author has no conflict of interest

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