Comparison of different Ki67 antibodies in human glioblastomas

S H Torp, E Johannesen, C F Lindboe

Abstract

***Aims*—To compare immunostaining between the original Ki67 monoclonal antibody and a new polyclonal Ki67 antibody on frozen and paraffin wax sections of human glioblastomas.**

***Methods*—Frozen sections and formalin fixed, paraffin wax embedded sections of the same tumour specimens were included in the study (10 cases). Half of the paraffin wax sections were pretreated in a microwave oven. Standard immunohistochemical techniques were used (avidin-biotin peroxidase complex). Five high power fields were examined using an eyepiece graticule, and 500 to 2000 tumour cells were counted. The labelling index was defined as the percentage of positive tumour cells.**

***Results*—The Ki67 monoclonal antibody displayed positive immunostaining in all frozen sections (median labelling index 5·9, range 2·6–11·4) whereas only four paraffin wax sections stained positively and only after pretreatment in a microwave oven. The polyclonal Ki67 antibody elicited positive staining in both frozen sections (median labelling index 13·7, range 6·7–21·5) and in paraffin wax sections (median labelling index 12·0, range 2·2–22·7) but only after pretreatment in a microwave oven.**

***Conclusion*—The Ki67 monoclonal antibody is not recommended for use on paraffin wax sections of glioma tissues whereas the new polyclonal Ki67 antibody provides satisfactory immunostaining on both frozen and paraffin wax sections.**

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Keywords: Ki67, polyclonal antibody, glioblastomas.

Estimation of proliferative activity in tumour tissues may be of prognostic importance, and the use of the Ki67 monoclonal antibody has proved to be a reliable and easy method for detecting proliferating cells in human tumours. This antibody reacts selectively with the nuclei of proliferating cells.[1 2] Many reports have demonstrated a positive correlation between Ki67 labelling index, tumour stage, tumour grade, and prognosis.[3]

The Ki67 labelling index correlates well with histological grade in human astrocytomas[4-8] whereas divergent results have been reported concerning the prognostic significance of Ki67 immunostaining in gliomas.[5 6 8-11]

The original Ki67 monoclonal antibody has been shown to work well on frozen sections, but with variable results on paraffin wax sections after pretreatment in a microwave oven.[12-14] A new generation of Ki67 antibodies for use on formalin fixed, paraffin wax embedded tissue sections has now been developed.[14 15]

The aim of this study was to correlate the immunostaining of the original Ki67 monoclonal antibody with a novel Ki67 polyclonal antibody on frozen and paraffin wax sections of human glioblastomas to determine whether proliferative activity can be adequately assessed on the latter.

Methods

Fresh tumour tissue was obtained from 10 patients with glioblastoma who underwent surgery during 1993 at the Department of Neurosurgery, University Hospital of Trondheim, Norway. The specimens were submitted to the Department of Pathology for frozen section examination. The fresh tumour samples were frozen in a microtome at −39°C, and frozen sections were cut and stored at −50°C until analysis. The remaining tumour tissue was thawed, fixed in 4% buffered formalin for approximately six hours, and embedded in paraffin wax for routine examination. The gliomas were graded according to the World Health Organisation criteria.[16]

Nine of 10 patients received steroids prior to surgery. Two patients underwent surgery for a second time because of tumour recurrence but had received radio- and/or chemotherapy after their first operation (table). The frozen sections were air dried, fixed in acetone for 10 minutes and dried in air again. Immunohistochemistry was performed using a commercial avidin-biotin peroxidase kit (Vectastain ABC kit, Vector Laboratories, Burlingame, California, USA). The sections were incubated with the Ki67 monoclonal antibody (Dako, Glostrup, Denmark)[1 2] at a 1 in 25 dilution and with the Ki67 polyclonal antibody (rabbit antihuman Ki67 antigen; Dako)[15] at a 1 in 150 dilution for one hour at room temperature.

The paraffin wax sections were dewaxed, rehydrated and stained using the same procedure as used for the frozen sections. Half of the deparaffinised sections were pretreated in a microwave oven prior to immunostaining (2 × 5 minutes, 600 W, citrate buffer (pH 6·0)). The sections were incubated with diaminobenzidine and were counterstained with haematoxylin. In each experiment sections of human tonsils were used as positive controls, while sections in which the primary antibodies had been omitted served as negative controls.

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Summary of Ki67 labelling indexes in human glioblastomas

Case No.	Sex/Age (years)	Site[a]	Preoperative treatment[b]	Ki67 (monoclonal)			Ki67 (polyclonal)		
				Frozen sections	Paraffin wax sections		Frozen sections	Paraffin wax sections	
					−MW	+MW		−MW	+MW
1	F/56	F	S	2·6	0	0	16·1	0	22·7
2	M/45	TO	S	7·9	0	4·0	21·5	0	12·5
3	M/56	T	S	3·7	0	1·7	12·8	0	11·5
4	M/83	F	S	2·9	0	0	13·4	0	10·7
5	M/60	T	S	4·7	0	0	16·1	0	16·9
6	F/81	F	S	5·1	0	0	9·2	0	3·8
7	M/68	T	S	11·4	0	0	14·0	0	12·7
8	M/66	CC	S	6·6	0	1·1	6·7	0	2·2
9[c]	F/30	T	RC	6·6	0	0	12·5	0	8·1
10[d]	M/43	T	SR	6·9	0	2·0	16·1	0	19·2
Median				5·9	0	0	13·7	0	12·0

[a] Tumour localisation: F = frontal; O = occipital; T = temporal; CC = corpus callosum.
[b] S = steroids; R = radiotherapy; C = chemotherapy.
[c] Astrocytoma grade II resected in 1988, postoperative radio- and chemotherapy, recurrence and reoperation in 1993 (glioblastoma) with no pretreatment.
[d] Astrocytoma grade II resected in 1990, preoperative steroid treatment, postoperative radiotherapy, recurrence and reoperation in 1993 (glioblastoma) with preoperative steroid treatment.
−/+MW = without/with pretreatment in a microwave oven.

The Ki67 labelling index for each tumour was determined by use of a standard graticule and manual counting system. Five randomly selected and representative fields were examined including 500 to 2000 cells per tumour. Areas of necrosis and endothelial cells were always excluded. The Ki67 labelling index was defined as the percentage of Ki67 positive cells. Tumours with indexes below 0·1% were given the value 0.

Results

The clinical data and Ki67 labelling indexes of the various groups are presented in the table. Both Ki67 antibodies demonstrated little background staining and positive immunostaining was easy to interpret. In paraffin wax sections pretreatment in a microwave oven was a prerequisite for positive immunostaining. With the Ki67 monoclonal antibody all frozen sections displayed positive immunostaining with a median labelling index of 5·9 (range 2·6–11·4) whereas only four of 10 paraffin wax sections were positive (median 0·0, range 0·0–4·0). The Ki67 polyclonal antibody stained all frozen and paraffin wax sections positively; the median labelling index in the frozen sections was 13·7 (range 6·7–21·5) and in the paraffin wax sections 12·0 (range 2·2–22·7). The labelling indexes obtained with the Ki67 monoclonal antibody were generally lower than those with polyclonal Ki67. There were satisfactory correlations between the labelling indexes determined with the monoclonal and polyclonal Ki67 antibodies on frozen sections ($r = 0·78$) and with the polyclonal antibody on frozen and paraffin wax sections ($r = 0·72$).

Discussion

In this study we have demonstrated excellent immunostaining on both frozen and routine paraffin wax sections of human glioblastomas with a novel Ki67 polyclonal antibody.

The original Ki67 monoclonal antibody has been useful on frozen sections only, which is of limited clinical use. However, this antibody has recently been tested on formalin fixed, paraffin wax embedded sections pretreated in a microwave oven with divergent results.[12–14] These discrepancies may be due to different microwave oven processing procedures.[13] In this study the standard protocol for pre-processing in a microwave oven comprised 2 × 5 minutes at 600 W. The Ki67 monoclonal antibody elicited variable staining results on microwave treated paraffin wax sections and only four out of 10 cases were regarded as positive. Thus, we conclude that this antibody is not suitable for use on paraffin wax sections, even after pretreatment in a microwave oven.

The Ki67 polyclonal antibody identified a greater number of proliferating cells than its monoclonal counterpart. We have no obvious explanation for this discrepancy, but it may be related to inherent differences between these antibodies (monoclonal *v* polyclonal) and reactivity against different epitopes on the Ki67 antigen.

The new Ki67 polyclonal antibody appears to be a reliable marker for proliferative activity in paraffin wax sections of human gliomas, but further studies are needed to clarify its prognostic value. Previous studies using the Ki67 monoclonal antibody have shown that determination of the Ki67 labelling index may be of prognostic significance in low grade astrocytomas[6] and for tumour grading in small stereotactically sampled biopsy specimens of astrocytic tumours.[7]

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