

Immunohistochemical Evaluation of Minichromosome Maintenance Protein 7 in Astrocytoma Grading

A. FACOETTI¹, E. RANZA¹, I. GRECCHI¹, E. BENERICETTI², M. CERONI^{3,4}, P. MORBINI⁵ and R. NANO^{1,4}

¹Dipartimento di Biologia Animale and ⁴Fondazione Istituto Neurologico "C.Mondino" IRCCS, Università di Pavia, Pavia;

²Divisione di Neurochirurgia, Azienda Ospedaliera Ospedale Maggiore di Parma, Parma;

³Divisione di Neurologia, Policlinico di Monza (MI);

⁵Dipartimento di Patologia, IRCCS Policlinico S. Matteo, Pavia, Italy

Abstract. The minichromosome maintenance (MCM) proteins, which play an important role in eukaryotic DNA replication, represent a group of proteins that are currently under investigation as novel diagnostic tumor markers. Several studies have proved a greater reliability of MCM proteins to stain proliferating cells compared to Ki67 protein, a routinely used proliferation marker in histopathology. In the present study, the expressions of MCM7 and Ki67 were estimated in 66 primary human astrocytomas in relation to tumor grade (Grade I-IV, WHO). MCM7 significantly stained more nuclei compared to Ki67 in all the histopathological grades investigated. In addition, a stronger increase of the MCM7 labelling index, in relation to the tumor aggressiveness, was observed.

Minichromosome maintenance (MCM) proteins represent a protein family, MCM2 to MCM7, which forms heterohexameric complexes at the replication origin during eukaryotic DNA duplication, licensing DNA replication (1). This makes MCM proteins attractive as histological markers (2), supported by increasing evidence of a better correlation between MCM7 expression and proliferating cells compared to Ki67 in many tumors (3-8).

Astrocytic tumors comprise a wide range of neuroectodermal tumors that differ in growth potential, extent of invasiveness, morphological features, tendency for progression and clinical outcome (9). According to WHO classification, these tumors are distinguished in pilocytic astrocytoma (PA; WHO grade I), diffuse astrocytoma (DA;

WHO grade II), anaplastic astrocytoma (AA; WHO grade III) and glioblastoma multiforme (GBM; WHO grade IV).

In a recent study (10) we assessed the utility of MCM7 protein as a marker of proliferation in glioblastoma. Additional evidence of the importance of MCM in astrocytoma proliferation measurement comes from the work of Scott *et al.* (11), who investigated astrocytomas grade II to IV.

Our study aims to compare the immunohistochemical staining patterns of Ki67 and MCM7 in the complete staging of astrocytoma, from grade I to grade IV (WHO), comprising a significant number of benign pilocytic astrocytomas.

Materials and Methods

Tissue specimens. Sixty-six archival formalin-fixed, paraffin-embedded human astrocytomas were obtained from diagnostic biopsy or resection specimens from 39 patients at the IRCCS Policlinico S. Matteo (Pavia, Italy). All astrocytomas included in this study were histopathologically diagnosed by neuropathologists and classified according to WHO classification as PA (n=27), DA (n=9), AA (n=14) and GBM (n=16).

Immunohistochemical staining. Immunohistochemical analysis with monoclonal anti-human Ki67 antibody (clone MIB-1, Dako) and monoclonal anti-human MCM-7 antibody (USBiological, Massachusetts, USA) was performed on tumor samples as previously described (10). Negative controls were performed by omitting the primary antibodies.

Quantification of immunohistochemical staining. The immunohistochemical stainings of MCM7 and Ki67 were independently determined by two investigators and the labelling index (LI) was calculated as the percentage of positively-stained nuclei out of the total number of nuclei counted in representative microscopic 40x objective fields. LI quantification was performed in the same area for each tumor sample, previously delineated by overlapping of the serial slides. The percentage of positive cells per 1,000 tumor cells was regarded as a LI.

Correspondence to: Angelica Facoetti, Department of Animal Biology, University of Pavia, P.zza Botta 10, 27100 Pavia, Italy. Tel: +39 0382 986405, Fax: +39 0382 986406, e-mail: facoetti@unipv.it

Key Words: Astrocytoma, MCM7, Ki67, proliferation markers, grading.

Statistical analysis. A paired *t*-test was performed to determine whether the differences were statistically significant. A *p*<0.05 was considered significant.

Results

Representative examples of immunostaining for Ki67 and MCM7 in the four tumor grades are provided in Figure 1. Positive immunoreactivity for Ki67 and MCM7, defined as positive nuclear staining of tumor cell in all cases of DA, AA and GBM was observed. In 2 cases out of 27 of PA, neither the Ki67 nor MCM7 antibody was able to detect any cell.

In the PA cases, the mean Ki67 index was 1.13%, in DA 3.04%, in AA 6.7% and in GBM 12.91%. The mean positive staining for MCM7 was seen in 2.94% of PA, 8.37% of DA, 13.8% of AA and 26.61% of GBM (Figure 2). By comparing these results, a significantly higher expression of MCM7 protein was clearly observed in all the astrocytoma grades compared to Ki67. In addition, MCM7 LI was significantly (*p*<0.05) higher in 20/27 (74%) of PA, 8/9 (89%) of DA, 14/15 (93%) of AA and 15/16 (94%) of GBM samples (Figure 2).

Discussion

The aim of this study was to evaluate the expression of MCM7 protein in all the histopathological grades of astrocytic tumors. Although there is increasing attention to these molecules in brain tumor (7, 8, 11), this is the first work examining the frequency and pattern of MCM7 protein expression in pilocytic astrocytoma, in addition to the malignant histologies. To assess its value as an aid for astrocytoma grading, MCM7 expression was compared with the Ki67 labelling index.

From this study it is evident that the number of Ki67-stained cells in all astrocytoma grades investigated was significantly underestimated compared to MCM7. This finding is similar to the data described for other malignancies (3-8) and it was hypothesized that the MCM7 antibody stains more cells than Ki67, because it is present in cells licensed to proliferate in addition to those that are already proliferating (12). Additionally, the MCM7 labelling index displayed significant relationship to tumor grade: it steeply increased with the tumor grade. This is consistent with other studies that have assessed this relationship in other malignancies, such as oligodendrogiomas (8), uterine (13) and colorectal cancer (14).

In conclusion, the present findings indicate that MCM proteins are good proliferation markers for astrocytic tumors, since they identified a larger proliferation fraction than Ki67 with a clear difference in the LI values among the malignant grades. This favours the inclusion of these

molecules in the histopathological analysis, although further data is now required to validate the clinical utility of MCM7 in predicting prognosis and eventually the response to adjuvant chemotherapy and radiotherapy in astrocytoma.

Acknowledgements

This work was partially funded by the Associazione Carlotta, Seregno, Milano, Italy.

References

- 1 Tye BK: MCM proteins in DNA replication. *Annu Rev Biochem* 68: 649-686, 1999.
- 2 Endl E, Kausch I, Baack M, Knippers R, Gerdes J and Scholzen T: The expression of Ki-67, MCM3, and p27 defines distinct subsets of proliferating, resting, and differentiated cells. *J Pathol* 195: 457-462, 2001.
- 3 Rodins K, Cheale M, Coleman N and Fox SB: Minichromosome maintenance protein 2 expression in normal kidney and renal cell carcinoma. *Clin Cancer Res* 8: 1075-1081, 2002.
- 4 Meng MV, Grossfeld GD, Williams GH, Dilworth S, Stoeber K, Mulley TW, Weinberg V, Carroll PR and Tlsty TD: Minichromosome maintenance protein 2 expression in prostate: characterization and association with outcome after therapy for cancer. *Cancer Res* 7: 2712-2718, 2001.
- 5 Gonzalez MA, Pinder SE, Callagy G, Vowler SL, Morris LS, Bird K, Bell JA, Laskey RA and Coleman N: Minichromosome maintenance protein 2 is a strong independent prognostic marker in breast cancer. *J Clin Oncol* 23: 4306-4313, 2003.
- 6 Ramnath N, Hernandez FJ, Tan DF, Huberman JA, Natarajan N, Beck AF, Hyland A, Todorov IT, Brooks JS and Bepler G: MCM2 is an independent predictor of survival in patients with non-small-cell lung cancer. *J Clin Oncol* 22: 4259-4266, 2001.
- 7 Hunt DP, Freeman A, Morris LS, Burnet NG, Bird K, Davies TW, Laskey RA and Coleman N: Early recurrence of benign meningioma correlates with expression of mini-chromosome maintenance-2 protein. *Br J Neurosurg* 1: 10-15, 2002.
- 8 Wharton SB, Chan KK, Anderson JR, Stoeber K and Williams GH: Replicative Mcm2 protein as a novel proliferation marker in oligodendrogiomas and its relationship to Ki67 labelling index, histological grade and prognosis. *Neuropathol Appl Neurobiol* 4: 305-313, 2001.
- 9 Kleihues P and Cavenee WK: Tumor of the Nervous System. Pathology and Genetics. World Health Organization International Classification of Tumours. IARC press. Lyon, pp. 9, 2000.
- 10 Facoetti A, Ranza E, Benericetti E, Ceroni M, Tedeschi F and Nano R: Minichromosome maintenance protein 7: a reliable tool for glioblastoma proliferation index. *Anticancer Res* 26: 1071-1076, 2006.
- 11 Scott IS, Morris LS, Rushbrook SM, Bird K, Vowler SL, Burnet NG and Coleman N: Immunohistochemical estimation of cell cycle entry and phase distribution in astrocytomas: applications in diagnostic neuropathology. *Neuropathol Appl Neurobiol* 5: 455-466, 2005.

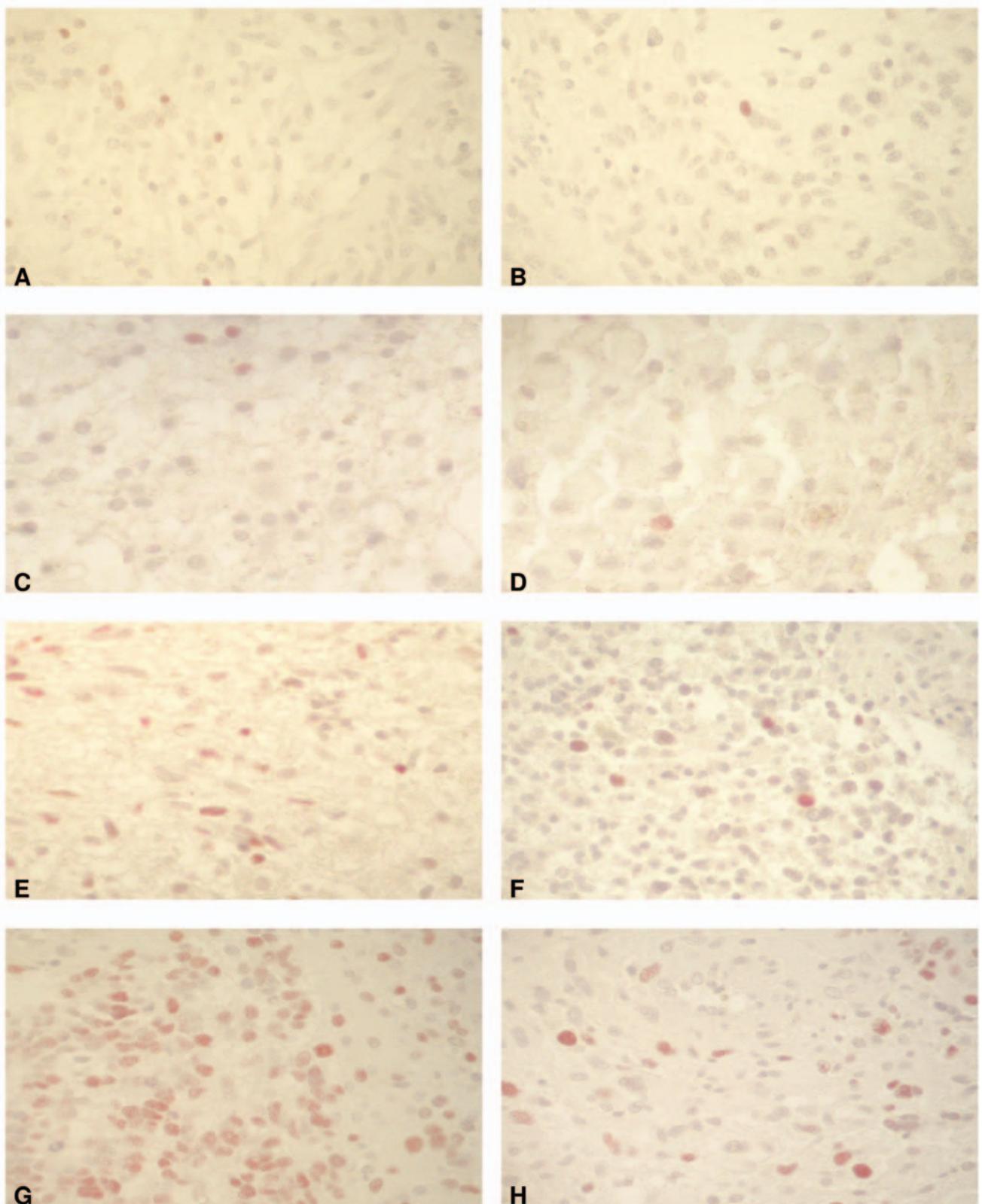


Figure 1. Representative immunohistochemical staining of MCM7 (left panels) and Ki67 (right panels) in pilocytic astrocytoma (A, B), diffuse astrocytoma (C, D), anaplastic astrocytoma (E, F) and glioblastoma multiforme (G, H). Original magnification $\times 400$.

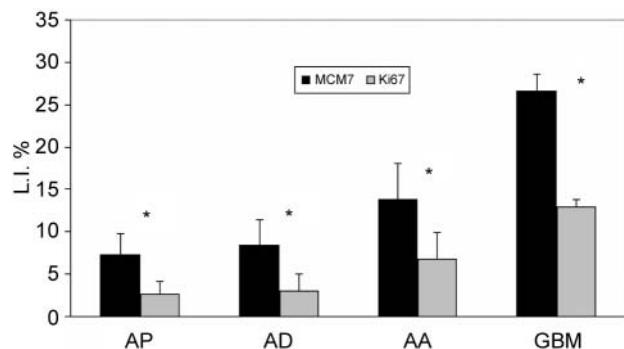


Figure 2. Distribution of MCM7 and Ki67 labelling index (LI) in human astrocytoma. Pilocytic Astrocytoma = PA; Diffuse Astrocytoma = DA; Anaplastic Astrocytoma = AA; Glioblastoma Multiforme = GBM.
*Difference statistically significant ($p < 0.05$).

- 12 Stoeber K, Tlsty TD, Happerfield L, Thomas GA, Romanov S, Bobrow L, Williams ED and Williams GH: DNA replication licensing and human cell proliferation. *J Cell Sci* 11: 2027-2041, 2001.
- 13 Ishimi Y, Okayasu I, Kato C, Kwon HJ, Kimura H, Yamada K and Song SY: Enhanced expression of Mcm proteins in cancer cells derived from uterine cervix. *Eur J Biochem* 6: 1089-1101, 2003.
- 14 Scott IS, Morris LS, Bird K, Davies RJ, Vowler SL, Rushbrook SM, Marshall AE, Laskey RA, Miller R, Arends MJ and Coleman N: A novel immunohistochemical method to estimate cell-cycle phase distribution in archival tissue: implications for the prediction of outcome in colorectal cancer. *J Pathol* 2: 187-197, 2003.

Received April 28, 2006

Accepted June 16, 2006