

13 April 2001
Original version submitted

MCM2- A promising marker for premalignant lesions of the lung: A cohort study

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MCM2—A Superior Marker For Premalignant Lesions of the Lung

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ABSTRACT

Background: Because cells progressing to cancer must proliferate, marker proteins specific to proliferating cells may permit detection of premalignant lesions. Here we compared the sensitivities of a classic proliferation marker, Ki-67, with a new proliferation marker, MCM2, in 41 bronchial biopsy specimens representing normal mucosa, metaplasia, dysplasia, and carcinoma *in situ*.

Methods: Parallel sections were stained with antibodies against MCM2 and Ki-67, and the frequencies of staining were independently measured by two investigators. Differences were evaluated statistically using the two-sided correlated samples t-test and Wilcoxon rank sum test.

Results: For each of the 41 specimens, the average frequency of staining by anti-MCM2 (39%) was significantly ($p < 0.001$) greater than by anti-Ki-67 (16%). In metaplastic lesions anti-MCM2 frequently detected cells near the epithelial surface, while anti-Ki-67 did not.

Conclusions: We conclude that MCM2 is detectable in 2-3 times more proliferating premalignant lung cells than is Ki-67. The superiority of MCM2 as a sensitive marker for premalignant lung cells is enhanced by the fact that it is present in cells at the surface of metaplastic lung lesions, which are more likely to be exfoliated into sputum. Future studies will determine if use of anti-MCM2 makes possible sufficiently early detection to significantly enhance lung cancer survival rates.

INTRODUCTION

The 5-year survival rate for patients with lung cancer is approximately 15%, and it has changed only marginally in the last 30 years [1]. Tumor stage is the most significant prognostic parameter for 5-year survival, but even patients with non-small cell lung cancer (non-SCLC) in pathologic stage IA disease (a tumor of less than 3 cm diameter located in one lobe of the lung and more than 2 cm from the carina without visceral pleural involvement, atelectasis, or pneumonitis, and absence of metastatic spread to regional lymph nodes) have a 33% chance of recurrence within 5 years after complete surgical resection (lobectomy with mediastinal lymph node dissection) [2]. In this group of patients, the tumor most frequently recurs at distant sites, including the bone, liver, adrenal glands, and brain [3], and the size of the primary tumor does not appear to impact on survival [4]. This suggests that even small and seemingly resectable lung cancers metastasize early. Data from randomized screening trials for lung cancer corroborate this observation. In these studies, more cancers were detected in resectable stages, and 5-year survival rates were higher in the screened population compared to the control population, but mortality rates (total death rate independent of time) from lung cancer were equal in both groups [5].

For this reason, it is important to develop methods that will permit facile detection of bronchial mucosal abnormalities that are precursors for lung cancer before systemic shedding of tumor cells occurs. Such precursor lesions can be detected by sputum cytology and by bronchoscopy in large airways accessible by endoscopy. They include metaplasia, dysplasia, and carcinoma *in situ* (CIS), which are thought to represent progressive histologic correlates of carcinogenesis for squamous cell carcinoma [6]. Current data suggest that 23% of current and former smokers have metaplastic lesions, and 2% have dysplastic lesions [7]. However, not all such lesions progress to lung cancer. For instance, smoking cessation, which can be viewed as a form of active intervention, appears to result in a decrease of metaplasia rates from 27% in active smokers to 7% in former smokers [7]. It is estimated that approximately 50% of CIS will progress to invasive cancer over a 6-month time period [8]. However, of 9 patients followed by regular bronchoscopy at 6-month time intervals, 4 developed lung cancer at sites that had previously been biopsied and interpreted as normal bronchial epithelium [8]. These results raise several important questions: A) Are there determinants in premalignant lesions that predict outcome, i.e., progression versus regression? B) Are there determinants in morphologically normal bronchial mucosa that

predict outcome? C) Can lung cancer arise directly from normal bronchial mucosa or are histopathologic intermediates required?

To address these questions, one promising approach would be the development of specific immunohistochemical markers capable of improving the sensitivity and reliability of methods currently employed to detect precursor lesions in histologic and cytologic specimens [9, 10]. Because proliferation is a requirement for lung cancer development, markers specific for cell proliferation are expected to prove useful.

Two proliferation markers, proliferating cell nuclear antigen (PCNA) and Ki-67, have been extensively studied in this context. PCNA is a homotrimeric protein that binds tightly to DNA and to proteins involved in DNA replication and repair. It is essential for DNA replication and is found in all proliferating cells. However, because PCNA is also essential for several types of DNA repair, it may be present in non-proliferating cells [11, 12]. Ki-67 is an epitope of a nuclear protein recognized by the MIB-1 monoclonal antibody. The protein is frequently expressed throughout the cell cycle of proliferating cells, and it has not been detected in non-proliferating cells. During interphase, Ki-67 is located primarily in nucleolar and peri-nucleolar regions, and it appears to be associated with condensed chromatin [13]. The function of the Ki-67 protein is still unknown [14]. Immunohistochemical studies with PCNA and Ki-67 indicate that, in at least some cases, increased lung tumor staining for these markers correlates with decreased survival [15-18]. These proliferation markers can also be detected in premalignant lesions of the lung [19-22].

In this report, we describe the results of our comparison of one of these classic proliferation markers, Ki-67, with a new proliferation marker, MCM2 [23]. MCM2 is one of six members of the minichromosome maintenance (MCM) protein family. These serve as components of "licensing factor," which is essential for initiation of DNA replication and for limiting replication to one round per cell cycle [24]. The MCM proteins are also associated with replication forks and are likely to stimulate the unwinding of the parental DNA strands at these forks [25]. We previously demonstrated [23] that, in normal tissues, MCM2 is detectable only in proliferating cells. Not surprisingly, it is also present in a high proportion of cancer cells. Our results suggested that it is a better marker for premalignant breast lesions than either PCNA or Ki-67 [23]. Others have similarly demonstrated the superiority of MCM family

proteins over Ki-67 and PCNA for detection of a variety of premalignant cell types [26, 27], but a comparison of MCM proteins with Ki-67 or PCNA as markers for premalignant lung lesions has not previously been reported.

METHODS

Study population

MCM2 and Ki-67 expression in bronchial biopsy specimens from patients at risk for lung cancer or with suspected lung cancer were studied by immunohistochemistry (IHC). We reviewed all pathology specimens from 106 patients that had undergone bronchoscopy with standard white light and laser-induced fluorescence at Roswell Park Cancer Institute from March 1998 to March 2000. Fourteen of these 106 patients were selected for further analysis based on the presence of abnormal morphology and the availability of multiple specimen blocks (4-17 per patient) for investigations. Of these 14 patients, the highest degree of abnormality in 4 was metaplasia, in 4 dysplasia, in 3 CIS, in 1 invasive squamous cell carcinoma, and in 2 invasive adenocarcinoma. Five of these patients were Caucasian women, 1 was an African-American man, and 8 were Caucasian men. All were active or former smokers.

Sample preparation and selection

From each specimen block, 5 serial 4- μ m sections were cut and placed on charged glass slides. Sections 1-4 were used for IHC, and section 5 was stained with hematoxylin and eosin. This latter section was reviewed and compared with the original slide used for diagnostic purposes. In 41 specimens, the morphology was comparable between the original slide and slide 5. These 41 specimens were used for IHC analysis and included morphologically normal bronchial mucosa, metaplasia, dysplasia, and CIS (see Table 1 for the numbers of specimens with normal and abnormal morphology).

Immunohistochemistry

Sections were stained with polyclonal rabbit antibodies raised and affinity-purified against the N-terminal portion of MCM2 [23]. Parallel sections were stained with the MIB-1 mouse monoclonal antibody against Ki-67 (DAKO, Carpinteria, California). IHC was performed as described earlier [23]. Briefly, sections were deparaffinized, and those to be stained with Ki-67 were subjected to microwave antigen retrieval in citrate buffer for 10 minutes, twice. Antigen retrieval was not required for MCM2. MIB-1 was used at a dilution of 1:50. The MCM2 antibody was used at a dilution of 1:500. The avidin-biotin detection method was employed on a Ventana Automated System (Tucson, Arizona). An irrelevant

rabbit antiserum served as a negative control. Percentage, intensity, and distribution of stained cells were analyzed and scored for each antibody independently by two of the authors (DFT, JAH) with similar results.

Statistical Analysis

The percent of cells stained for each sample was estimated as a continuous number ranging from 0 to 100. The staining intensity was categorically coded as 0 (absent), 1 (weak), 2 (moderate), or 3 (strong). The average of both the percent of cells stained and staining intensity was calculated for each type of specimen (normal mucosa, metaplasia, dysplasia, CIS). To test for differences between the staining characteristics of MCM2 and Ki-67 both the parametric paired samples t-test and the non-parametric Wilcoxon rank-sum test were performed. To test for differences across specimen classes (normal mucosa, metaplasia, dysplasia and CIS) for each stain (Ki-67 or MCM2), the analysis of variance method as well as the non-parametric Kruskal-Wallis tests were used. To test specifically for differences between dysplasia and CIS for each stain, the independent samples t-test and the Mann-Whitney tests were performed. Both parametric and non-parametric tests were used because the distributional assumptions required for parametric testing may not be satisfied in all cases. All statistical tests assumed a two-sided alternative with a 5% level of significance.

RESULTS

In normal bronchial mucosa, antibodies against both proteins, MCM2 and Ki-67, generated similar patterns: some cells in the basal and parabasal layer of the bronchial epithelium displayed immunoreactivity (Fig. 1). The proportion of cells staining for MCM2 was significantly ($p<0.02$) greater than for Ki-67 (Table 1). In both cases, the intensity of nuclear staining was variable.

In premalignant lesions, evaluation of the staining by the two antibodies (Table 1) revealed significantly ($p<0.001$) more frequent staining of nuclei within metaplastic lesions by antibodies against MCM2 than by antibodies against Ki-67. We also noticed that, in metaplasia, anti-MCM2 frequently stained both basal cells and cells throughout the entire thickness of the epithelium, while anti-Ki-67 primarily stained basal cells and cells in the lower half of the epithelium (Fig. 1, lower of the two “metaplasia” panels). Although the number of samples of dysplasia and CIS was too low to permit statistical evaluation of the differences between the two antibodies, we noticed that in every case of dysplasia or CIS, the frequency of cells stained by anti-MCM2 was as high or higher than the frequency of staining by anti-Ki-67. In fact, the same relationship was evident in the normal mucosa and metaplasia samples. Consequently, when the data for all 41 samples were combined, the difference in staining frequency between anti-MCM2 and anti-Ki-67 was highly significant ($p<0.001$; Table 1).

We observed variable staining in premalignant lesions. In many cases, within single lesions some regions stained while others did not, and the intensity of staining was variable from region to region and from cell to cell within regions. The level of variability was similar for both markers (Fig. 1).

In the progression from normal mucosa to metaplasia to dysplasia, the results obtained with both antibodies suggested differences in staining frequency and intensity. For both antibodies, the mean percentages of cells stained and staining intensity were different across specimen categories (all p -values ≤ 0.010) and increased from normal mucosa to metaplasia and from metaplasia to dysplasia. Although the sample numbers are too low to draw a firm conclusion, the data suggest a possible decrease in staining frequency by both antibodies in the transition from dysplasia to CIS ($p=0.117$ for Ki-67 and $p=0.080$ for MCM2) (Table 1).

No significant differences were detected between the average stain intensities produced by the two antibodies (Fig. 1; Table 1).

DISCUSSION

This is the first comparison of MCM2 and Ki-67 expression in premalignant lesions of the human lung, and it showed that MCM2 was expressed in a greater percentage of cells in normal mucosa and in premalignant lesions than Ki-67. The proportion of cells stained for MCM2 ranged from 5% to 100% and for Ki-67 from 1% to 60% (Table 1). The average percentage of cells stained for MCM2 (11-64%) was higher than that for Ki-67 (3-34%). Statistical analyses of the frequencies of positively stained cells were done with the paired samples t-test and the Wilcoxon sign rank test. Both tests showed a striking difference ($p < 0.001$) in immunoreactivity between these markers across all 41 samples. This finding suggests that MCM2 will prove to be a better marker than Ki-67 for easy, reliable detection of premalignant lesions of the lung.

There are two reasons why MCM2 is likely to be a better marker than Ki-67 for premalignant lesions of the lung. First, the fact that anti-MCM2 more frequently stains metaplastic cells at the epithelial surface suggests that it is more likely than anti-Ki-67 to detect exfoliated premalignant cells in cytological specimens such as sputum. Second, the proportion of metaplastic cells stained by anti-MCM2 is significantly ($p < 0.001$) higher than the proportion stained by anti-Ki-67. This increased frequency (average 39% for anti-MCM2 compared to 14% for anti-Ki-67) means that the chance of detecting metaplastic cells using MCM2 as a marker is nearly 3-fold higher than the chance when using Ki-67 as a marker.

The cellular function of the Ki-67 protein is not yet known [14]. In contrast, the requirement for MCM2 in DNA replication is well established [24, 25]. Thus all proliferating cells must contain MCM2. Previous data suggest that MCM2 is expressed only in proliferating cells [23, 26, 27]. Therefore, our observation that significantly fewer cells in premalignant lung lesions stain with anti-Ki-67 than with anti-MCM2 suggests that the Ki-67 protein may not be present in all proliferating cells and may not be essential for cellular proliferation.

CONCLUSION

We have confirmed that MCM2 immunoreactivity provides consistent and reliable staining in routinely fixed tissues without a requirement for antigen retrieval. Results obtained are easy to interpret, since there is a striking difference between normal bronchoepithelium and premalignant lesions. Thus MCM2 is an easy-to-use marker, which has great potential for assessment of progression and regression of morphologically abnormal lesions in future primary lung cancer prevention studies. In addition, the number of MCM2-positive cells in normal bronchial epithelium may provide insight into the proliferative activity and potential progression from normal tissue to invasive cancer without morphologically abnormal intermediates. MCM2 immunoreactivity thus provides an improved tool which can be employed in future studies to address the questions raised earlier: A) Is MCM2 a determinant in premalignant lesions predictive of outcome, i.e., progression versus regression? B) Is MCM2 a determinant in morphologically normal bronchial mucosa predictive of outcome? C) Can lung cancer arise directly from normal bronchial mucosa or are histopathologic intermediates required?

ACKNOWLEDGEMENTS

This research was supported in part by a Research Award from the Roswell Park Alliance Foundation to DFT, a grant (GM49294) from the National Institute of General Medicine to JAH, and a Developmental Funds Award from the Roswell Park Cancer Center Support Grant (P30 CA16056-24) and the Roswell Park Alliance Foundation to GB.

REFERENCES

1. Greenlee RT, Murray T, Bolden S, Wingo PA: **Cancer statistics, 2000**. *CA Cancer J Clin* 2000, **50**:7-33.
2. Mountain C: **Revisions in the international system for staging lung cancer**. *Chest* 1997, **111**:1710-1717.
3. Martini N, Ginsberg R: **Treatment of stage I and II disease**. In: *Comprehensive textbook of thoracic oncology* Edited by Aisner J, Arriagada R, Green M, Martini N, Perry M. pp. 339-350. Baltimore: Williams and Williams; 1996: 339-350.
4. Patz EF, Jr., Rossi S, Harpole DH, Jr., Herndon JE, Goodman PC: **Correlation of tumor size and survival in patients with stage IA non-small cell lung cancer**. *Chest* 2000, **117**:1568-71.
5. Patz E, Goodman P, Bepler G: **Early detection and screening for lung cancer**. *N Engl J Med* 2000, **in press**.
6. Saccomanno G, Archer VE, Auerbach O, Saunders RP, Brennan LM: **Development of carcinoma of the lung as reflected in exfoliated cells**. *Cancer* 1974, **33**:256-70.
7. Morice R, Lee J, Kurie J, Khuri F, Ro J, Kemp B, Broxson A, Liu D, Hong W: **Bronchial squamous metaplasia and dysplasia in current and former smokers**. *Proc Am Soc Clin Oncol* 1999, **18**:472a.
8. Venmans B, van Boxem T, Smit E, Psotmus P, Sutedja T: **Outcome of bronchial carcinoma *in situ***. *Chest* 2000, **117**:1572-1576.
9. Tockman MS, Gupta PK, Myers JD, Frost JK, Baylin SB, Gold EB, Chase AM, Wilkinson PH, Mulshine JL: **Sensitive and specific monoclonal antibody recognition of human lung cancer antigen on preserved sputum cells: a new approach to early lung cancer detection**. *J Clin Oncol* 1988, **6**:1685-93.
10. Bechtel JJ, Petty TL, Saccomanno G: **Five year survival and later outcome of patients with X-ray occult lung cancer detected by sputum cytology [In Process Citation]**. *Lung Cancer* 2000, **30**:1-7.
11. Kelman Z, Hurwitz J: **Protein-PCNA interactions: a DNA-scanning mechanism?** *Trends Biochem. Sci.* 1998, **23**.
12. Tsurimoto T: **PCNA, a multifunctional ring on DNA**. *Biochim. Biophys. Acta* 1998, **1443**:23-39.
13. Kreitz S, Fackelmayer FO, Gerdes J, Knippers R: **The proliferation-specific human Ki-67 protein is a constituent of compact chromatin**. *Exp. Cell Res.* 2000, **261**:284-292.

14. Endl E, Gerdes J: **The Ki-67 protein: fascinating forms and an unknown function.** *Exp Cell Res* 2000, **257**:231-7.
15. Lavezzi AM, Santambrogio L, Bellaviti N, Biondo B, Nosotti M, Radice F, Maturri L: **Prognostic significance of different biomarkers in non-small cell lung cancer.** *Oncol Rep* 1999, **6**:819-25.
16. Fukuse T, Hirata T, Naiki H, Hitomi S, Wada H: **Expression of proliferating cell nuclear antigen and CD44 variant isoforms in the primary and metastatic sites of nonsmall cell lung carcinoma with intrapulmonary metastases.** *Cancer* 1999, **86**:1174-81.
17. Oyama T, Osaki T, Nose N, Ichiki Y, Inoue M, Imoto H, Yoshimatsu T, Kodate M, Uramoto H, Mizoue T, Yano K, Yasumoto K: **Evaluations of p53 immunoreactivity, nucleolar organizer regions, and proliferating cell nuclear antigen in non-small cell lung carcinoma.** *Anticancer Res* 2000, **20**:505-10.
18. Shiba M, Kohno H, Kakizawa K, Iizasa T, Otsuji M, Saitoh Y, Hiroshima K, Ohwada H, Fujisawa T: **Ki-67 immunostaining and other prognostic factors including tobacco smoking in patients with resected nonsmall cell lung carcinoma.** *Cancer* 2000, **89**:1457-65.
19. Pendleton N, Dixon GR, Burnett HE, Occleston NL, Myskow MW, Green JA: **Expression of proliferating cell nuclear antigen (PCNA) in dysplasia of the bronchial epithelium.** *J Pathol* 1993, **170**:169-72.
20. Tormanen U, Nuorva K, Soini Y, Paakko P: **Apoptotic activity is increased in parallel with the metaplasia-dysplasia-carcinoma sequence of the bronchial epithelium.** *Br J Cancer* 1999, **79**:996-1002.
21. Kitaguchi S, Takeshima Y, Nishisaka T, Inai K: **Proliferative activity, p53 expression and loss of heterozygosity on 3p, 9p and 17p in atypical adenomatous hyperplasia of the lung.** *Hiroshima J Med Sci* 1998, **47**:17-25.
22. Yokose T, Ito Y, Ochiai A: **High prevalence of atypical adenomatous hyperplasia of the lung in autopsy specimens from elderly patients with malignant neoplasms.** *Lung Cancer* 2000, **29**:125-30.
23. Todorov IT, Werness BA, Wang HQ, Buddharaju LN, Todorova PD, Slocum HK, Brooks JS, Huberman JA: **HsMCM2/BM28: a novel proliferation marker for human tumors and normal tissues.** *Lab Invest* 1998, **78**:73-8.

24. Kearsley SE, Maiorano D, Holmes EC, Todorov IT: **The role of MCM proteins in the cell cycle control of genome duplication.** *Bioessays* 1996, **18**:183-90.
25. Labib K, Tercero JA, Diffley JFX: **Uninterrupted MCM2-7 function required for DNA replication fork progression.** *Science* 2000, **288**:1643-1647.
26. Freeman A, Morris LS, Mills AD, Stoeber K, Laskey RA, Williams GH, Coleman N: **Minichromosome maintenance proteins as biological markers of dysplasia and malignancy.** *Clin Cancer Res* 1999, **5**:2121-32.
27. Williams GH, Romanowski P, Morris L, Madine M, Mills AD, Stoeber K, Marr J, Laskey RA, Coleman N: **Improved cervical smear assessment using antibodies against proteins that regulate DNA replication.** *Proc Natl Acad Sci U S A* 1998, **95**:14932-7.

FIGURE LEGEND

Fig. 1. Comparisons of staining by anti-MCM2 and anti-Ki-67 in parallel sections of normal bronchial mucosa and premalignant lung lesions. All objective lens magnifications were 20x except for the upper panels of normal bronchial mucosa and metaplasia, which were 40x.

Table 1. Percentage and intensity of staining for MCM2 and Ki-67 in normal bronchial mucosa and premalignant lesions

Type of Specimen	Number of Specimens	% Cells Positive for MCM2 Mean (Range)	% Cells Positive for Ki-67 Mean (Range)	Mean Intensity for MCM2 ^a	Mean Intensity for Ki-67 ^a
Normal Mucosa	7	11 ^{b,e} (5-20)	3 ^{b,f} (1-5)	1.6 ⁱ (1.0-2.0)	1.6 ⁱ (1.0-2.5)
Metaplasia	21	39 ^{c,e} (7-90)	14 ^{c,f} (1-60)	2.3 ⁱ (1.0-3.0)	2.3 ⁱ (1.0-3.0)
Dysplasia	7	64 ^{d,e,g} (40-100)	34 ^{d,f} (7-40)	2.6 ⁱ (2.0-3.0)	2.8 ⁱ (2.0-3.0)
CIS	6	39 ^{e,g} (5-60)	17 ^{f,h} (3-60)	2.4 ⁱ (2.0-3.0)	2.3 ⁱ (1.0-3.0)
Combined data	41	39 ^c (5-100)	16 ^c (1-60)	2.2 (1.0 – 3.0)	2.3 (1.0-3.0)

^aThe intensity of stain ranges from 0 (absent) to 3 (strong).

^b p-value <0.016 for the paired difference t-test and Wilcoxon sign rank test.

^c p-value <0.001 for the paired difference t-test and Wilcoxon sign rank test.

^d p-value <0.034 for the paired difference t-test and <0.063 for the Wilcoxon sign-rank test.

^e p-value <0.010 for the ANOVA and Kruskal-Wallis tests of differences in MCM2 category means.

^f p-value <0.001 for the ANOVA and Kruskal-Wallis tests of differences in Ki-67 category means.

^g p-value <0.080 for the independent samples t-test between dysplasia and CIS within MCM2.

^h p-value <0.117 for the independent samples t-test between dysplasia and CIS within Ki67.

ⁱ p-value <0.004 for the ANOVA and Kruskal-Wallis tests of differences in MCM2 and Ki-67 category means.

8 May 2001
Reviewers' reports

MCM2- A promising marker for premalignant lesions of the lung: A cohort study

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Elmar Endl

Reviewing of the manuscript by Bepler et al. now entitled "MCM2 - A promising marker for premalignant lesions of the lung" was done very well by the authors. The authors responded to every point raised by the referees and their arguments are justified. I would therefore recommend the manuscript for publication.

It may sound petty-minded, but I would appreciate one minor modification of the manuscript. On page 14 line 7 the authors try to explain why there is a difference in the expression of the Ki-67 and the MCM2 protein. The sentences "It is thus intriguing to speculate that Ki-67 may be predominantly expressed during G1, S, G2, or M phase", "If Ki-67 were present only in G1 phase ..." (p. 14, l. 9) and "Conversely, if Ki-67 were present only in G2 phase.." (p. 14, l. 11) are hard to understand. The Ki-67 protein is definitely present in S, G2 and M phase and during a certain time period during G1 and so is the MCM2 protein. Any differences in the expression of both proteins should therefore arise from a different schedule for up and down regulation during the G1 phase. I might have missed the point, but it may also be irritating for readers that are not so familiar with the topic. However I agree with the authors that staining for both proteins will provide more information about the current status of cells within the cell cycle not obtainable for one marker alone. I leave it to the editor to decide whether this modification is necessary and to correct any final version.

As a remark, there is a typing error in the references that were added during the review process. The first author of reference number 15 (P. 18, l. 15) is spelled Schluter not Schulter (Or more correct Schlueter, due to the Umlaut that is not displayed in PubMed).

Nevertheless, the revision resulted in a well rounded manuscript that adds a new topic that should be discussed within the community.

Level of interest: A paper whose findings are important to those with closely related research interests

Advice: Accept without revision

Quality of written English: Acceptable

Competing interests: none declared.

Giovanni Bussolati

Comments:

The study reports on the use of a new proliferation marker, MCM2, in bronchial biopsies representative of different histological lesions. The data on the percentage of nuclei stained, obtained with this marker, were compared with those obtained with the commonly used Ki67 marker. The result indicate that MCM2 is superior in terms of number of stained nuclei, compared with Ki67. The technique is correctly explained.

The figures are satisfactory.

The study reproduces in part the data reported on the literature on the higher percentage of positive nuclei obtained with this marker, compared to the result of Ki67 staining. However previous studies did not specifically focus on lung lesion. Moreover, the present investigation might find diagnostic application, in the diagnosis of metaplastic changes of the bronchial mucosa. The authors seem not to consider the fact that the vast majority of metaplastic lesions of the bronchi are reactive (e.g. in chronic bronchitis). It cannot be excluded that metaplastic lesions are precancerous, but their neoplastic potential is limited, unless displasia is associated.

Despite these criticism, I regard the paper as sufficiently original and potentially helpful so that I recommend it for publication.

I suggest:

1. To omit the second half of the conclusion (page 14), which is totally theoretical.
2. The antibody used is a policlonal serum (not commercially available). This is going to limit the reproducibility of the study in other laboratories. I would recommend to test, if possible, a monoclonal antibody (BM28) against MCM2, now commercially available.

Level of interest

A paper whose findings are important to those with closely related research interests

Advice on publication

Accept after revision, which I do not need to see

Quality of written English

Acceptable

Competing interests

None declared

Have you in the past five years received reimbursements, fees, funding, or salary from an organisation that may in any way gain or lose financially from the publication of this paper? If so, please specify.

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Do you hold any stocks or shares in an organization that may in any way gain or lose financially from the publication of this paper? If so, please specify.

No

Do you have any other financial competing interests? If so, please specify.

No

Are there any non-financial competing interests you would like to declare in relation to this paper? If so, please specify.

No

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