RESEARCH ARTICLE

DNA Replication Licensing Proteins for Early Detection of Lung Cancer

Veena VS¹, Rajan K², Saritha VN³, Preethi Sara George⁴, Chandramohan K⁵, Jayasree K¹, Thara S¹, K Sujathan³*

Abstract

Background: To identify and characterize malignant and premalignant cells in sputum and matched tissue samples with reference to expression of minichromosome maintenance proteins (MCM2, MCM5) and cell division cycle protein 6 (CDC 6) and to assess their potential as biomarkers of premalignant and malignant lesions of the lung and associations with clinicopathological features. Methods: Expression of MCM2, MCM5 and 6 proteins in sputum samples and corresponding tissues was assessed by immunocytochemistry, and correlated with histological findings. Results: For characterization of malignant, metaplastic or dysplastic cells, CDC6 protein had the highest sensitivity of 87.7%. All the three markers together had a sensitivity of 94.4%. Furthermore these proteins could be employed to assess the proliferative potential of precancerous or atypical cells, as overexpression increasing with the stage of disease and degree of metastasis. Conclusion: The assessed markers can be utilized in routine cytopathology laboratories to supplement conventional morphological evaluation so that the sensitivity of sputum cytology can be enhanced. Potential applications in predicting the clinical behavior of lung lesions and predicting prognosis and survival deserve further attention.

Keywords: Minichromosome maintenance proteins- cell division cycle protein (CDC6)- sputum- immunocytochemistry

Asian Pac J Cancer Prev. 18 (11), 3041-3047

Introduction

Despite recent advances in prevention, screening and treatment modalities, non-small cell lung cancer (NSCLC) remains as the leading cause of cancer related mortality worldwide, resulting in 1.6 million deaths each year and has a very poor survival rate, which has been attributed to the late diagnosis (Stewart and Christopher, 2014). Even in patients with stage I A tumors, there is a chance of recurrence in 33% of cases within 5 years after complete surgical resection (Martini et al., 1995). If early detection of lung cancer can be achieved by awareness programmes and more sensitive screening modalities, a longer average survival can definitely be offered (Ramnath et al., 2001).

Lung carcinogenesis is a multistep process characterized by the sequential accumulation of successive molecular, genetic and epigenetic abnormalities. Along with this, a series of morphological alterations of normal bronchial or bronchioloalveolar epithelium occurs, resulting in preneoplastic and neoplastic lesions. The major mucosal changes in the large airways that may precede or accompany invasive squamous cell carcinoma include hyperplasia (basal cell hyperplasia and goblet cell hyperplasia), squamous metaplasia, different grades of dysplasia (mild, moderate and severe) and carcinoma in situ (Brambilla et al., 2001). Atypical adenomatous hyperplasia is considered as a preneoplastic condition of bronchioalveolar carcinoma, and diffuse idiopathic pulmonary neuroendocrine cell hyperplasia is a proposed precursor of carcinoid tumors (Greenberg et al., 2002).

Sputum cytology has been recognized as the only noninvasive laboratory method of diagnosis for lung cancer, but it has very low sensitivity. The lack of adequate number of cells is one of the main reasons for its poor sensitivity (Palcic et al., 2002). Moreover, the reactive changes caused by different laboratory processing methods cause the cells to appear so atypical that distinguishing it as malignant / premalignant or benign is often difficult and can be rectified to an extent by robust techniques that can fish out the whole cell content of sputum samples. If a marker protein can be characterised to supplement the morphological evaluation, identification of malignant and premalignant cells becomes easier.

Abnormal cell proliferation, resulting from deregulation of the cell cycle is fundamental in tumorigenesis. The integrated mechanism that regulates the accurate replication of DNA and correct division of cells has a pivotal role in the neoplastic process (Evan and Vousden, 2001).
Veena Vs et al
Asian Pacific Journal of Cancer Prevention, Vol 18

from each participant. (HEC No.31/2014) and informed consent was obtained from the study group. The study was approved by the Institutional review board and Human ethics committee of pulmonary macrophages less than 5) were excluded and subjects with inadequate sputum samples (number of pulmonary macrophages less than 5) were excluded from the study group. The study was approved by the Institutional review board and Human ethics committee (HEC No.31/2014) and informed consent was obtained from each participant.

Materials and Methods

Subjects

The subjects for the study were selected from a cohort of 3185 patients referred from the Sanatorium for Chest Disease and Medical College Hospital from 2010-2015. All these patients were with chronic obstructive pulmonary disease and/or radiologic findings suspicious of malignancy. The subjects included both genders in the age group of 30-76 years. Among them, 90 cases had histologically proven malignant lesions which include 33 adenocarcinoma, 23 squamous cell carcinoma, 25 non-small cell carcinoma and 9 small cell carcinoma. These cases were selected based on the adequacy of their sputum samples, availability of corresponding tissue samples and satisfactory clinical follow-up data. Among them, 5 cases were in stage 1, 16 cases were in stage 2, 29 cases in stage 3A and 15 cases were in stage 3B and 25 cases in stage 4. In addition, 57 subjects were also selected based on sputum cytology. Among them 16 samples had atypical cells suspicious of malignancy, 20 samples with metaplastic cells and 21 cases with no evidence of malignancy. The clinical complaints and other clinico-pathological details were collected from patients’ records and documented on a proforma. Subjects with any history of treatment for cancer or any such chronic ailments such as tuberculosis and subjects with inadequate sputum samples (number of pulmonary macrophages less than 5) were excluded from the study group. The study was approved by the Institutional review board and Human ethics committee (HEC No.31/2014) and informed consent was obtained from each participant.

Sample collection

Sputum samples were selected based on morphological evaluation and matched bronchoscopic biopsy samples were collected for comparison as a gold standard. Sputum samples were collected for 3-5 consecutive days, homogenized and processed using red solution (Cytorich® red Preservative Tripath Imaging Inc. Burlington NC, 27215, USA). The samples were vortexed with twice the volume of red solution and kept for 30 minutes. The mixed sample was then transferred to a 50 mL centrifuge tube and again vortexed, centrifuged at 600 g for 5 minutes. The pellet was re-suspended in buffer solution and again vortexed, centrifuged at 800 g for 10 minutes. The cell pellet was again vortexed and monolayer smear was prepared by using the settling chamber assembly provided by BD Surepath on pre-coated slides and the remaining samples were used for cellblock preparation (Veena et al., 2015; Sujathan et al., 2000).

Immunocytochemistry was performed in 5 micron sections from cell blocks/monolayered smears and corresponding tissue samples according to standard ABC technique using DAB as chromogen. Sections were incubated with primary antibody for overnight and Novalink polymer was used as secondary system. Antigen retrieval was done by microwave technique in sodium citrate buffer (pH 6.0) at 700W for 15 mints. Primary antibodies were procured from Santha Cruz Laboratories (CDC 6 mouse monoclonal antibody, dilution 1:50, Positive control-tonsil tissue) and Novacastra (MCM2 and MCM5, mouse monoclonal antibody, dilution 1:25, Positive control-tonsil tissue). In monolayer smears, cell permeability was enhanced by treating with Sodium deoxycholate. Western blot analysis was performed for all the markers to assess the sensitivity of the antibodies. Immunoscoring was performed by two of the investigators independently. A repeat scoring was performed for samples having any dispute in diagnosis for sputum. Nuclear staining was considered as specific of malignancy. The immunopositivity of tumor cells was assessed by counting a minimum of 200 cells from at least 3 representative high power fields. The H scores were then calculated as the product of intensity (0-3) and distribution (0-100 %) with H-score ranging from 0-300 and H-score 30 and above was taken as positive.

Analysis of MCM and CDC6 proteins and statistics

Statistical analysis was performed using SPSS-11 software. Sensitivity and specificity of each of the markers were assessed along with positive and negative predictive value taking histology report as gold standard. The comparison of expression pattern of all the 3 markers in cytology and histology samples was done by paired t-test.

Results

The correlation of the clinicopathological features with protein expression revealed that MCM2 proteins have significant association with tumor stage (p = 0.04) only (Table 1). Whereas, MCM 5 protein showed significant association with tumor stage (p = 0.03), histologic type
focal expression was noticed in a few cells. The majority of metaplastic cells also showed mild expression in a few samples. On the other hand, atypical cells showed dark or intense staining, but in a limited number of cells. The intensity of staining in different regions of same lesion also found to vary in tissue sections.

MCM2 proteins were found to have a sensitivity of 58.89% (95% confidence Interval (CI): 48.02%-69.16%) and specificity of 73.68% (95% CI: 60.33% to 84.45%) for a diagnosis of malignancy. MCM5 proteins showed a sensitivity of 66.67% (95% CI: 55.94 to 76.2%) and a specificity of 70.18% (95 CI: 56.60% and 81.5%). CDC6 was found to have a sensitivity of 87.78% (95% CI: 79.18% to 93.7%) and specificity of 70.18% (95% CI: 56.6% to 81.5%). (Table 3). The MCM2 and MCM5 proteins together had a sensitivity of 73.33% (95% CI: 62.97% to 82.10%) and a specificity of 59.56% (95% CI: 45.82% to 72.43%).

Adenocarcinoma samples showed the highest H score of 66.7, 95 and 97.9 for MCM2, MCM5 and CDC6 respectively (Table 2). Mild, moderate or dense expression of MCM 2 (Figure 1 A-H), MCM 5 (Figure 2A-H) and CDC6 (Figure 3 A-H) were found in the nuclei of tumor cells and their expression patterns were similar in both sputum cell blocks, corresponding tissue samples as well as in the smears. The intensity of expression showed a slight variation between monolayered smears and cellblocks compared to tissue samples. The CDC6 proteins expressed weak positivity in the cytoplasm also in a few of the tumor cells. The samples designated as negative for malignancy had no expression for all the three markers, except for a few samples in which mild focal expression was noticed in a few cells. The majority of metaplastic cells also showed mild expression in a few samples. On the other hand, atypical cells showed dark or intense staining, but in a limited number of cells. The intensity of staining in different regions of same lesion also found to vary in tissue sections.

MCM2 proteins were found to have a sensitivity of 58.89% (95% confidence Interval (CI): 48.02%-69.16%) and specificity of 73.68% (95% CI: 60.33% to 84.45%) for a diagnosis of malignancy. MCM5 proteins showed a sensitivity of 66.67% (95% CI: 55.94 to 76.2%) and a specificity of 70.18% (95 CI: 56.60% and 81.5%). CDC6 was found to have a sensitivity of 87.78% (95% CI: 79.18% to 93.7%) and specificity of 70.18% (95% CI: 56.6% to 81.5%). (Table 3). The MCM2 and MCM5 proteins together had a sensitivity of 73.33% (95% CI: 62.97% to 82.10%) and a specificity of 59.56% (95% CI: 45.82% to 72.43%). The MCM2 and CDC6 proteins together had a sensitivity of 93.33% (95% CI: 86.04%
Table 2. Mean H -Score / Std.Deviation of Different Markers for Different Lung Lesions

| Sample                  | Diagnosis                  | Number of Specimens | MCM 2 Mean H-score (Std.deviation) | MCM5 Mean H-score (Std.deviation) | CDC6 Mean H Score (Std.deviation) |
|-------------------------|----------------------------|---------------------|-----------------------------------|----------------------------------|-----------------------------------|
| Sputum                  | Negative for Malignancy    | 21                  | 12 (14.5)                         | 10.9 (14.2)                      | 14.8 (27.6)                      |
| Sputum                  | Metaplastic cells          | 20                  | 38.0 (51.3)                       | 32.6 (39.2)                      | 35.1 (40.6)                      |
| Sputum                  | Atypical cells             | 16                  | 53.1 (47.9)                       | 54.4 (43.0)                      | 63.1 (45.6)                      |
| Sputum/Cell block/tissue| ADC                        | 33                  | 66.7 (54.5)                       | 95.0 (65.8)                      | 97.9 (62.6)                      |
| Sputum/Cell block/tissue| SCC                        | 23                  | 52.6 (51.5)                       | 64.1 (45.6)                      | 79.6 (59.5)                      |
| Sputum/Cell block/tissue| NSCLC                      | 25                  | 45.2 (33.9)                       | 68.4 (55.4)                      | 64 (48)                          |
| Sputum/Cell block/tissue| SCLC                       | 9                   | 31.1 (32.6)                       | 35.6 (41.6)                      | 86.1 (63.0)                      |

Table 3. Sensitivity and Specificity of MCM2, MCM5 and CDC6 Based on H- Score

| Markers        | Sensitivity (%) | Specificity (%) | Positive predictive value (%) | Negative predictive value (%) |
|----------------|-----------------|-----------------|-------------------------------|-------------------------------|
| MCM2           | 58.89 (48.02-69.16) | 73.68 (60.33-84.45) | 77.94 (66.24-87.09) | 53.16 (41.60-64.49)         |
| MCM5           | 66.67 (55.94-76.25)  | 70.18 (56.60-81.56) | 77.92 (67.02-86.58) | 57.14 (44.75-68.91)         |
| CDC6           | 87.78 (79.18-93.73)  | 70.18 (56.60-81.56) | 82.29 (73.17-89.33) | 78.43 (64.67-88.70)         |
| MCM2 and MCM5  | 73.33 (62.97-82.10)  | 59.65 (45.82-72.43) | 74.16 (63.79-82.86) | 58.62 (44.93-71.40)         |
| MCM2 and CDC6  | 92.33 (86.04-97.50)  | 59.65 (45.82-72.43) | 78.5 (69.51-85.86) | 85 (70.15-94.25)            |
| MCM5 and CDC6  | 94.44 (87.5-98.15)   | 50.88 (37.29-64.37) | 75.22 (66.22-82.86) | 85.29 (68.93-94.99)         |
| MCM5, MCM2 and CDC6 | 94.44 (87.50-98.15) | 49.12 (35.63-62.71) | 74.56 (65.55-82.25) | 84.85 (68.09-94.83)         |

Figure 1. Figure 1A-H: A. Mild nuclear expression of MCM 2 in metaplastic cells (Sputum - 40x). B. Moderate nuclear expression of MCM 2 in atypical metaplastic cells (Sputum - 40x). C. Dense expression of MCM 2 in squamous cell carcinoma cells (Sputum cell block- 40x). D. Nuclear expression of MCM 2 in squamous cell carcinoma, occasional cells show dense staining and few cells show diffuse staining (Tissue-40x). E. Intense nuclear expression of MCM 2 in squamous cell carcinoma cells (Sputum -40x). F. Dense nuclear expression of MCM 2 in adenocarcinoma cells (Sputum-40x). G. Intense nuclear expression of MCM 2 in small cell carcinoma cells (Sputum-40x).

Figure 2. A-H: A. Mild nuclear expression of MCM 5 in metaplastic cells (Sputum - 40x). B. Moderate nuclear expression of MCM 5 in atypical metaplastic cells (Sputum- 40x). C. Dense nuclear expression of MCM 5 in squamous cell carcinoma cells (Tissue- 40x). D. Dense nuclear expression of MCM 5 in squamous cell carcinoma cells (Sputum-40x). E. Intense nuclear expression of MCM 5 in adenocarcinoma cells (Tissue-40x). F. Dense nuclear expression of MCM 5 in adenocarcinoma cells (Sputum-40x). G. Intense nuclear expression of MCM5 in non small cell carcinoma cells (Sputum-40x). H. Diffuse nuclear expression of MCM 5 in small cell carcinoma cells (sputum-40x).
to 97.5%) and a specificity of 59.65% (95% CI: 45.82% to 72.43%). The combination of MCM5 and CDC6 together had a sensitivity of 94.44% (95% CI: 87.50% to 98.15%) and a specificity of 50.88 % (95% CI: 37.29% to 64.37%) (Table 3). All the three markers together showed a sensitivity of 94.4% for the identification of malignant cells. MCM5 and CDC6 together also had the same sensitivity suggesting that these markers can have a higher sensitivity when used in combination. It has been well demonstrated in many tumors that no particular MCM protein appears to be up-regulated in isolation, as it functions as a hexameric complex i.e. MCM 2-7 and that may be the reason for the higher sensitivity when used in combination. Studies employing these proteins in samples of uterine cervix have found to be advantageous for cervical cancer screening in low resource setting (Mukherjee et al., 2007). Moreover MCM 5 and other members of the MCM family of proteins, including MCM 2 and MCM 7 have been shown to be potentially useful markers for the detection of cervical lesions in tissue samples (Brake et al., 2003). The current study also observed immuno positivity in dysplastic cells and atypical cells compared to metaplastic cells in both sputum samples and tissue samples.

Even though analysis of the expression patterns of MCM2, MCM5 and CDC6 can be employed for assessing lung cancer risk and identifying precancerous lesions, some ambiguity exists while dealing with atypical cells or metaplastic cells. Most importantly, proliferation of cells, even though a hallmark of malignancy, also occurs as a component of inflammation and healing. Thus, metaplastic lesions usually originate in bronchial epithelium as a result of chronic irritation either by smoking or by chronic nonspecific inflammation and such lesions are present in 27% of current smokers and only 7% of former smokers (Morice et al., 1999). Furthermore, all high grade dysplasias need not necessarily lead to invasive cancer (Venmans et al., 2000) and it is often difficult to differentiate metaplastic cells with potential for progression from metaplastic cells originating due to inflammatory reactive changes.

The staining in the atypical / dysplastic areas vary greatly in intensity and pattern of staining from mild, diffuse staining to moderate, dense or intense staining.

**Discussion**

MCM proteins are expressed in abundance in all phases of cell cycle, but are degraded in quiescent, senescent or differentiated cells and these proteins are present only in replicating cells (Stoeber et al., 2001; Gonzalez et al., 2005). Most of the cells in malignant and premalignant lesions are in dividing stage so there will be marked accumulation MCM proteins on it. Gonzalez and Tachbana (2005) has demonstrated a substantial increase in the number of cells expressing MCM proteins in malignant and premalignant lesions of differentiating epithelia (Tachibana et al., 2005). Freeman have reported that MCM proteins are more frequently detected in cells from malignant tissues than normal tissues suggesting the role of MCM proteins as a good indicator of proliferative potential of neoplastic tissues (Freeman et al., 1999). As the precancerous cells and malignant cells are proliferating continuously, expression of these proteins will be enhanced in these lesions and this will help to filter out these cells from their normal counterparts. In the present study, the MCM proteins showed weak positivity in normal and metaplastic cells, but intense expression was observed in atypical and malignant cells. All the three markers together showed a sensitivity of 94.4% for the identification of malignant cells. MCM5 and CDC6 together also had the same sensitivity suggesting that these markers can have a higher sensitivity when used in combination. Studies employing these proteins in samples of uterine cervix have found to be advantageous for cervical cancer screening in low resource setting (Mukherjee et al., 2007). Moreover MCM 5 and other members of the MCM family of proteins, including MCM 2 and MCM 7 have been shown to be potentially useful markers for the detection of cervical lesions in tissue samples (Brake et al., 2003). The current study also observed immuno positivity in dysplastic cells and atypical cells compared to metaplastic cells in both sputum samples and tissue samples.
This progressive variation can be explained by the proliferative behaviour of the lesions. As the MCM proteins are replication licensing proteins, they can be detected abundantly in continuously proliferating cells. The mean percentages of cells stained and intensity of staining were different across specimen categories and increased from normal mucosa to metaplasia and to dysplasia for all the marker proteins used in this study. In addition to their potential for detecting frank malignancy, the progressive increase in the expression pattern of these markers may also provide an estimate of the nature of potentially precancerous conditions (metaplasia and dysplasia) in the patient’s airways. So it can be assumed that cells expressing intense and dense staining pattern may have the potential for proliferating into advanced lesions. This observation may be helpful in determining whether further investigations are needed for this patient to rule out any abnormal lesions in the lungs. This is very significant information for defining these proteins as markers for screening purpose. However, further studies with regular follow up and experimental demonstration in animal models are required to establish whether the progressive increase in the expression pattern correlates with potential of these lesions for progressing into malignancy.

Most of the previous studies in MCM proteins by immunohistochemistry (IHC) were performed in tissue samples and a very few reports were available analysing the role of MCM proteins as a predictive marker for lung cancer and precursor lesions in sputum samples. One of the studies detected these proteins in the peripheral blood of CML patients (Cai et al., 2015). To our knowledge this is the first study carried out in sputum samples and compared the expression pattern to that of corresponding tissue samples. The MCM proteins were abundantly present in cells at the surface of the metaplastic lung lesions which were more likely to be exfoliated into the sputum (Tan et al., 2001). So sputum samples can be an ideal platform for analysing premalignant lung lesions using MCM proteins. MCM-2 has been previously demonstrated as a sensitive marker for premalignant lung lesions in lung tissues and reported to be present in a greater percentage of cells than normal mucosa (Tan et al., 2001). It was also noticed that, majority of cells are MCM immunopositive in malignant and high grade premalignant lesions. As the dysplastic and malignant cells were continuously licensed for DNA replication and the presence of MCM proteins is mandatory for DNA replication. So these marker proteins are the ideal candidates for identifying premalignant and malignant lesions in the respiratory epithelium. As per the human protein atlas, lung cancer cells express this protein on a moderate intensity. (http://www.proteinatlas.org). Our study also supports this observation.

We have analysed the sensitivity of MCM proteins in sputum samples as a predictive marker for premalignant and malignant lesions. As MCM-positive cells usually appear at the surface of the abnormal epithelia, the cells exfoliating from this area i.e. cytology samples have higher sensitivity than histology samples (Stoeber et al., 2002; Davies et al., 2002). MCM proteins are therefore very promising biomarkers for early detection of malignancy and premalignancy in cytology samples. MCM positive cells in cytological preparations are easily identifiable due to crisp nuclear details; even at low magnification than histology samples (Williams et al., 1998; Chatrath et al., 2003) and these properties make MCMs highly reliable for detecting abnormal cells in cytological samples. This is particularly important when abnormal cells are rare, following sampling of a small lesion or only a small part of a larger lesion. MCM-based tests consequently show high sensitivity for detecting malignancy and premalignancy and can reduce the rate of false-negative results associated with conventional cytological screening (Andrew et al., 2014). The cytology samples obtained from sputum had the same expression pattern and sensitivity as that of tissue samples. So the current study suggests that these markers can be tried for population screening programme for lung cancer.

Another aspect revealed in our study was the significant association of MCM 2 with tumor stage and MCM 5 proteins with tumor stage, histological type of tumor and metastasis. This observation can be employed as a predictor of prognosis. These findings support the previous studies, which suggested that MCM7 markers can be used to predict tumor progression and prognosis of NSCLC patients (Toyonaka et al., 2011). Yang et al., (2006) also reported a significant association MCM2 expression with poor prognosis in patients with NSCLC, which suggests that identifying higher tumor proliferation may have an important role in predicting prognosis of NSCLC.

In conclusion, the present study has characterised the malignant cells, metaplastic cells and dysplastic cells of respiratory epithelium with MCM and CDC 6 proteins. This information can be utilised in routine cytopathology laboratories to supplement the conventional morphological evaluation so that the sensitivity of sputum cytology can be enhanced. The significant association of over expression of these proteins with the stage of disease and metastasis had potential application in predicting the clinical behaviour of lung lesions.

Acknowledgements

The authors acknowledge the department of Biotechnology, Govt. of India for the financial support for this study.

References

Andrew P. Jackson, Ronald A, Laskey, Coleman N (2014). Replication proteins and human disease. Cold Spring Harb Perspect Biol, 6, a013060.

Brake T, Connor JP, Petereit DG, Lambert PF (2003). Comparative analysis of cervical cancer in women and in a human papillomavirus-transgenic mouse model: identification of minichromosome maintenance protein 7 as an informative biomarker for human cancer. Cancer Res, 63, 8173-80.

Brambilla E, Travis WD, Colby TV, et al (2001). The new WHO classification of lung tumours. Eur Respir J, 18, 1059–68.

Cai L, Zhao K, Yuan X (2015). Expression of minichromosome proteins 8 in chronic myelogenous leukemia. Int J Clin Exp
Chaturath P, Scott IS, Morris LS, et al (2003). aberrant expression of minichromosome maintenance protein-2 and Ki67 in laryngeal squamous epithelial lesions. Br J Cancer, 89, 1048–54.

Davies RJ, Freeman A, Morris LS, et al (2002). Analysis of minichromosome maintenance proteins as a novel method for detection of colorectal cancer in stool. Lancet, 59, 1917–9.

Evans GL, Voussen KH (2001). Proliferation, cell cycle and apoptosis in cancer. Nature, 411, 342–8.

Freeman A, Morris LS, Mills AD, et al (1999). Minichromosome maintenance proteins as biological markers of dysplasia and malignancy. Clin Cancer Res, 5, 2121–32.

Gonzalez MA, Tachibana KK, Laskey RA, Coleman N (2005). Control of DNA replication and potential clinical exploitation. Nat Rev Cancer, 5, 135–41.

Greenberg AK, Yee H, Rom WN (2002). Preneoplastic lesions of the lung. Respir Res, 3, 20.

Kearsey SE, Maieronano D, Holmes EC, Todorov IT (1996). The role of MCM proteins in the cell cycle control of genome duplication. Bioessays, 18, 183-90.

Lopez-Saez JF, de la Torre C, Pincheira J, Gimenez-Martin G (1998). Cell proliferation and cancer. Histol Histopathol, 13, 1197-214.

Madine MA, Swietlik M, Pelizon C, et al (2000). The roles of the MCM, ORC, and Cdc6 proteins in determining the replication competence of chromatin in quiescent cells. J Struct Biol, 129, 198–210.

Moric R, Lee J, Kurie J, et al (1999). Bronchial squamous metaplasia and dysplasia in current and former smokers. Proc Am Soc Clin Oncol, 18, 472a.

Mukherjee G, Muralidhar B, Bafna UD, Laskey RA, Coleman N (2007). MCM immunocytochemistry as a first line cervical screening test in developing countries: a prospective cohort study in a regional cancer centre in India. Br J Cancer, 96, 1107 –11.

Martini N, Bains MS, Burt ME, et al (1995). Incidence of local recurrence and second primary tumors in resected stage I lung cancer. J Thorac Cardiovasc Surg, 109, 120-9.

Palcic B, Garner DM, Beveridge J, et al (2002). Increase of serous effusions: a combined approach to morphological features in papanicolaou and May-grunwald geimsa stained smears and a modified cell block technique. J Cytol, 17, 89–95.

Tachibana K, Gonzalez MA, Coleman N, et al (2005). Cell-cycle-dependent regulation of DNA replication and its relevance to cancer pathology. J Pathol, 205, 123–9.

Tan D, Huberman JA, Hyland A, et al (2001). MCM2 - a promising marker for premalignant lesions of the lung: a cohort study. BMC Cancer, 1, 6.

Todorov IT, Atarara A, Kearsey SE (1995). BM28, a human member of the MCM2-3-5 family, is displaced from chromatin during DNA replication. J Cell Biol, 129, 1433-45.

Toyokawa G, Masuda K, Daigo Y, et al (2011). Minichromosome Maintenance Protein 7 is a potential therapeutic target in human cancer and a novel prognostic marker of non-small cell lung cancer. Mol Cancer Biol, 10, 65.

Veena VS, Preethi SG, Jayasree K, Sujathan K (2015). Comparative analysis of cell morphology in sputum samples homogenized with dithiothreitol, N-acetyl-L cysteine, cytorych red preservative and in cellblock preparations to enhance the sensitivity of sputum cytology for the diagnosis of lung cancer. Diagcytopathol, 43, 551-8.

Vennmans B, van Boxtm T, Smit E, Psotmus P, Sutedja T (2000). Outcome of bronchial carcinoma in situ. Chest, 117, 1572-6.

Williams GH, Romanowski P, Mills AD, et al (1998). Improved cervical smear assessment using antibodies against proteins that regulate DNA replication. Proc Natl Acad Sci, 95, 14932–7.

Yang J, Ramnath N, Moysich KB, et al (2006). Prognostic significance of MCM2, Ki-67 and gelsolin in non-small cell lung cancer. BMC Cancer, 6, 203.

DOI:10.22034/APJCP.2017.18.11.3041

DNA Replication Licensing Proteins Lung Cancer Early Detection Markers

Asian Pacific Journal of Cancer Prevention, Vol 18 3047

DOI: 10.22034/APJCP.2017.18.11.3041

DNA Replication Licensing Proteins Lung Cancer Early Detection Markers

Pathol, 8, 14180–8.

Chatrath P, Scott IS, Morris LS, et al (2003). aberrant expression of minichromosome maintenance protein-2 and Ki67 in laryngeal squamous epithelial lesions. Br J Cancer, 89, 1048–54.

StoeberK, Tlsty TD, Happerfield L, et al (2001). DNA replication to once per cell cycle: the role of Cdc 6 and licensing and human cell proliferation. J Cell Sci, 114, 2027–41.

Stoeber K, Swinn R, Prevost AT, et al (2002). Diagnosis of genito-urinary tract cancer by detection of minichromosome maintenance 5 protein in urine sediments. J Natl Cancer Inst, 94, 1071–9.

Sujathan k, Pillai KR, Kannan S, et al (2000). Cyto-diagnosis of serious effusions: a combined approach to morphological features in papanicolaoue and May-grunwald geimsa stained smears and a modified cell block technique. J Cytol, 17, 89–95.