

Prognostic Value Of hMLH1 and hMSH2 Immunohistochemical Expression In Non-Small Cell Lung Cancer

J Skarda, E Fridman, P Plevova, M Hajduch, E Ofek, J Kopolovic, Z Kolar

Citation

J Skarda, E Fridman, P Plevova, M Hajduch, E Ofek, J Kopolovic, Z Kolar. *Prognostic Value Of hMLH1 and hMSH2 Immunohistochemical Expression In Non-Small Cell Lung Cancer*. The Internet Journal of Pulmonary Medicine. 2005 Volume 6 Number 1.

Abstract

Purpose and Experimental Design: The etiologic association and prognostic significance of mismatch repair gene/protein alterations have never been examined in lung cancer. We investigated protein expression of hMLH1 and hMSH2 genes in tumor specimens from 105 non-small cell lung cancer (NSCLC) patients. 60 of them were diagnosed with adenocarcinoma, 38 with squamous cell carcinoma and seven with large cell carcinoma. Out of the 105 patients 40 (20 adenocarcinomas and 20 squamous cell carcinomas) had already received neoadjuvant chemotherapy based on Carboplatine and navelbine or carboplatine vepesid. Immunohistochemical staining was used to examine protein expression. Expression in each patient was compared with clinicopathologic variables as well as overall survival and cancer-specific survival rates.

Results: Alteration of protein expression was observed in 30% of patients. Loss of hMLH1 and hMSH2 protein expression was associated with significantly shorter disease-free survival in patients who had received neoadjuvant chemotherapy ($p=0.1$).

Conclusion: Loss of immunohistochemical expression of hMLH1 and hMSH2 markers in lung tumors indicates a poorer prognosis for NSCLC patients receiving neoadjuvant therapy.

INTRODUCTION

Lung cancer has become the leading cause of cancer death in many industrialized countries. In Taiwan, for example, lung cancer claims more than 7,000 lives annually [13]. Much attention has recently been focused on the rapidly increasing incidence of primary lung cancer in nonsmokers [14, 21, 28]. Gender differences in distribution, histological type, and exposure to tobacco have also been noted [4, 6, 8, 16, 18]. Although 80% of female lung cancer patients worldwide have smoked at some time, less than 10% of Taiwanese women with lung cancer have any smoking history. The low smoking status and high incidence rate of adenocarcinoma constitute distinctive characteristics of lung cancer in Taiwanese females. Ryberg et al. showed that susceptibility to DNA damage caused by environmental carcinogens such as polycyclic aromatic hydrocarbon-like compounds may be higher among women than among men. They concluded that women are at greater risk of tobacco and/or

environmentally-induced lung cancer [19]. Takagi et al. observed a distinct mutational spectrum for the p53 gene in lung cancer tissue from nonsmoking Chinese women in Hong Kong suggesting that environmental and/or genetic factors might be involved in the development of lung cancer in these women [23].

Molecular biological studies have shown that overt cancers carry multiple genetic and epigenetic alterations, which seem to indicate the involvement of tumor suppressor genes and dominant oncogene activation during the process of carcinogenesis and subsequent progression of cancer [20, 25]. Alteration analysis of genes controlling acquired somatic mutations, such as genes involved in DNA repair, may explain the observed susceptibility to various environmental factors seen in lung cancer in nonsmoking females. hMLH1 and hMSH2 are both known to play a role in DNA mismatch repair. Their inactivation by promoter hypermethylation has been reported to be associated with some human cancers

[5,7,12,15,24]. Herman et al. have suggested that DNA methylation associated with transcriptional silencing of hMLH1 is the underlying cause of mismatch repair defects in most sporadic colorectal cancers [12]. Xinarianos et al. have shown that 58.6% and 57.8% of lung cancer tumor specimens had reduced expression levels of the hMLH1 and hMSH2 proteins, respectively [29].

The clinical significance of protein expression of hMLH1 and hMSH2 in lung cancers remains unclear. Recently, Brooks et al. reported that low protein expression of hMSH2 in positive mediastinal nodal specimens was associated with poor treatment response and cancer death in patients with stage III non-small cell lung cancer (NSCLC) [2]. In a previous study protein expression and status of promoter hypermethylation of hMLH1 and hMSH2 in 77 NSCLC tumors was studied. They found that protein expression in specimens from female patients was higher than in specimens from male patients, although relatively few female samples were included in the study (20).

In the present study we investigated protein expression in hMLH1 and hMSH2. A special focus was placed on the clinical association and prognostic significance of both genes in NSCLC.

MATERIALS AND METHODS

STUDY POPULATION AND TUMOR SAMPLES

105 patients diagnosed with NSCLC and operated on between 1996 and 2001 were enrolled in this study. There was complete follow-up for all patients. The end of the follow-up period was defined as October 2005. Overall survival was calculated from the day of surgery to the date of death or the last follow-up. Cancer-specific survival was calculated from the day of surgery to the date of either lung cancer death or the last follow-up. The mean follow-up period for all patients was 32 months (range: 0.5-78 months). Of 105 patients 40% (42) patients died from lung cancer and had a median cancer-specific survival of 17 months (range: 0.5-44 months).

ANALYSIS OF PROTEIN EXPRESSION: IMMUNOHISTOCHEMISTRY ASSAY

Paraffin blocks of tumors were cut into 5- μ m slices and then processed using protocols described previously [26]. hMLH1 and hMSH2 protein expression was evaluated by immunohistochemistry. The monoclonal antibodies used for the hMLH1 protein were G168-728 (1:250; PharMingen, San Diego, CA) and for the hMSH2 protein FE11 (1:50;

Oncogene Science, Cambridge, MA). The normal staining pattern for hMLH1 and hMSH2 is nuclear. Absence of nuclear staining in tumor cells along with positive staining in infiltrating lymphocytes was considered negative.

STATISTICAL ANALYSES

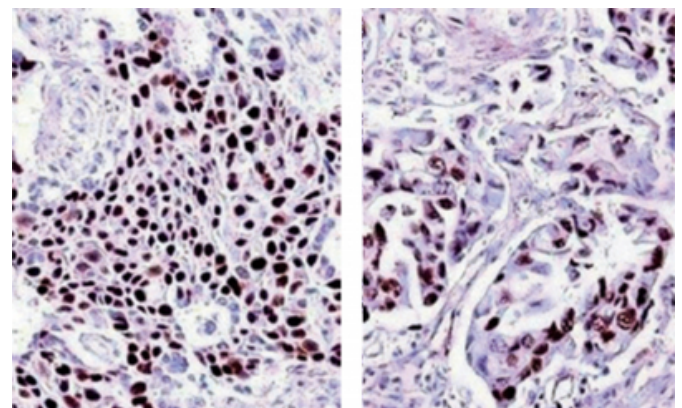
The Pearson χ^2 test was used to compare the hMLH1 and hMSH2 alterations among cases and between various clinicopathologic variables. The difference in age distribution between patients with and without the alteration was analyzed by the independent sample t test. Type III censoring was done on subjects who were still alive at the end of the study. Censoring for the cancer-specific survival analysis was done at the end of the study on subjects who were still alive or who had died of another cause. Survival curves were calculated by the Kaplan-Meier method, and comparison was done by the log-rank test. $P < \text{or} = 0.1$ was considered statistically significant.

RESULTS

Genetic alterations of hMLH1 protein/gene and its correlation with clinicopathologic variables in patients with lung cancer. Immunohistochemical staining for hMSH2 and hMLH1 proteins was done on 105 tumor samples. Nuclear staining within tumor cells was considered positive. Seventy percent of tumor specimens showed moderate to strong staining of both proteins (Fig. 1A and 1B). The remaining 30 percent showed a complete absence of nuclear staining. There was no significant correlation between the protein expression and patient age, smoking habit, tumor cell type, or tumor stage.

Figure 1

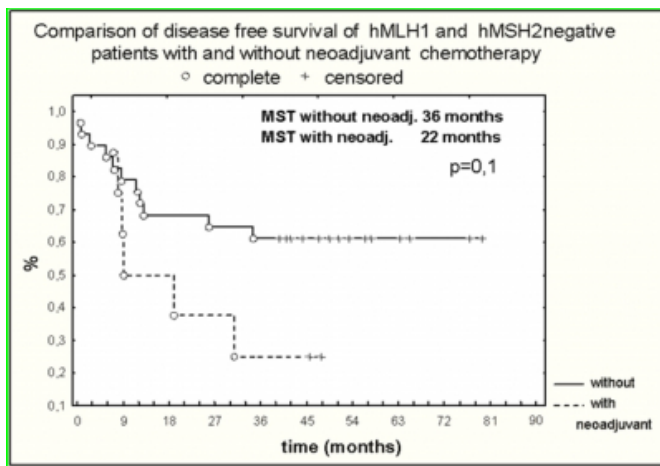
Figure 1: Representative figures of the immunohistochemical analysis of hMLH1 (A) and hMSH2 (B) protein expression in paraffin sections of lung tumor specimens. hMLH1 and hMSH2 nuclear immunoreactivity was found in (A) and (B). Original magnification, x100



Correlation of hMLH1 and hMSH2 alterations with the prognosis in non-small cell lung cancer patients. The relationship between survival and the alteration of hMLH1 and hMSH2 was analyzed (Fig. 2). Significantly shorter disease free survival was observed in of hMLH1 and hMSH2 patients with neoadjuvant chemotherapy as compared to the group of patients without neoadjuvant chemotherapy

Figure 2

Figure 2: Correlation of hMLH1 and hMSH2 alterations with the prognosis of non-small cell lung cancer patients. The relationship between survival and the alteration of hMLH1 and hMSH2 was analyzed. Significantly shorter disease free survival was observed in of hMLH1 and hMSH2 patients with neoadjuvant chemotherapy as compared to the group of patients without neoadjuvant chemotherapy.



DISCUSSION

In this study, we investigated the significance of the DNA mismatch repair genes, hMLH1 and hMSH2 in NSCLC using immunohistochemistry. Protein expression alteration was observed in 30% to 34% of tumor specimens for the hMLH1 and hMSH2 proteins and a significant concordance was observed between alteration in protein expression and disease free survival in patients with a history of neoadjuvant chemotherapy.

Data on mismatch repair gene alterations in lung cancer is scarce. Xinarianos et al. studied 59 males and 91 females with lung cancer in the United Kingdom and showed reduced expression levels of hMLH1 and hMSH2 proteins in 53% and 82% of adenocarcinoma specimens respectively [29]. These results are similar to ours except for the higher expression level of hMSH2 protein in their study. On the other hand, an immunohistochemical study by Aubry et al. showed that mismatch repair proteins hMLH1, hMSH2, and

hMSH6 were not inactivated in 33 bronchioloalveolar carcinomas of the lung [1]. This type of inconsistency also occurred in a U.S. study, which found no promoter methylation of the hMLH1 gene in 20 NSCLC tumors in a methylation-specific PCR (MSP) assay [9]. In a panel of 21 small cell lung cancer cell lines using the MSP assay Hansen et al. showed that low hMLH1 protein expression was not linked to promoter methylation [11]. The discrepancies between the various studies may be due to differences in clinicopathological variables. In addition, the methylation regions examined and sensitivities of the assays used in the various studies were different. It is also possible that geographic and/or ethnic factors may account for frequent hMLH1 and hMSH2 alterations in NSCLC patients.

Some authors have suggested that a relationship exists between the expression of hMLH1 and hMSH2 proteins and cancer drug resistance or response [2,13,17]. Strathee et al. investigated the role of methylation of hMLH1 in drug resistance in ovarian cancer cell lines and suggested that methylation of the hMLH1 promoter may be a common mechanism for cisplatin resistance in ovarian cancer [22]. In addition, Mackay et al. reported that reduction of hMLH1 protein expression in breast tumor samples after chemotherapy was strongly associated with poor disease-free survival [17]. Brooks et al. investigated expression of hMSH2 in positive mediastinal nodal specimens in 59 NSCLC patients with stage III disease treated with chemotherapy and irradiation and found that low expression of hMSH2 was associated with poor overall survival [2]. To the best of our knowledge, ours study is the first to show a relationship between the status of hMLH1 or hMSH2 expression and disease free survival of NSCLC patients with neoadjuvant therapy.

The association between DNA inactivation of mismatch repair genes and genetic instability has been described in some cancers. Loss of hMLH1 expression has proven to be one of the main causes of microsatellite instability in colorectal cancer [7,24]. Selective defects in some mismatch repair genes may cause genomic instability and activate malignant transformation as well as progression of gastric cancer, renal cell carcinoma, and endometrial carcinoma [5,10,15]. Highly significant correlation has also been reported between gene methylation and negative protein expression in hMLH1 and hMSH2, suggesting that promoter hypermethylation is the predominant mechanism by which these two mismatch repair genes are silenced in NSCLC [3,20]. Genetic instability may soon follow resulting in a

poorer prognosis in such cases.

Interestingly, Ward et al. [27,] have reported that the poor prognostic effect of DNA methylation is lost in colorectal patients with microsatellite instability. It seems that methylated tumors, both unstable and stable, have distinct clinicopathologic features. Further analysis regarding lung cancer is necessary. Mechanisms involving DNA damage signaling, promoter hypermethylation of mismatch repair genes, and target drug-resistant genes after chemotherapy should also be further investigated.

FOOTNOTES

This work has been dedicated to grant number IGA MZ čR NR/8425-3/2005 of the Ministry of Health in Czech Republic.

CORRESPONDENCE TO

Josef Skarda, MD Department of Pathology, Medical Faculty, Palacky University, Hnevotinska 3, CZ-77515, Olomouc, Czech Republic jojso@email.cz Tel/Fax.: 004-2060-3436129

Eduard Fridman, MD Department of Pathology, Chaim Sheba Medical Center, Sackler School of Medicine Tel-Aviv University, Tel-Hashomer 52621 Israel ef@sheba.health.gov.il Tel.: 972-3-5302354 Fax: 972-3-5302381

References

1. Aubry MC, Halling KC, Myers JL, Tazelaar HD, Yang P, Thibodeau SN (2001) DNA mismatch repair genes hMLH1, hMSH2, and hMSH6 are not inactivated in bronchioloalveolar carcinomas of the lung. *Cancer* 92: 2898-901.
2. Brooks KR, To K, Joshi MB, Conlon DH, Herndon JE, D'Amico TA, Harpole DH Jr. (2003) Measurement of chemoresistance markers in patients with stage III non-small cell lung cancer: a novel approach for patient selection. *Ann Thorac Surg* 76: 187-93.
3. Chang JW, Chen YC, Chen CY, Chen JT, Chen SK, Wang YC (2000) Correlation of genetic instability with mismatch repair protein expression and p53 mutations in non-small cell lung cancer. *Clin Cancer Res* 6: 1639-46.
4. Chiu YL, Yu TS, Wong TW (2004) Time trends of female lung cancer in Hong Kong: age, period and birth cohort analysis. *Int J Cancer* 111: 424-30.
5. Deguchi M, Shiina H, Igawa M, Kaneuchi M, Nakajima K, Dahiya R. (2003) DNA mismatch repair genes in renal cell carcinoma. *J Urol* 169: 2365-71.
6. de Perrot M, Licker M, Bouchardy C, Usel M, Robert J, Spiliopoulos A. (2000) Sex differences in presentation, management, and prognosis of patients with non-small cell lung carcinoma. *J Thorac Cardiovasc Surg* 119: 21-6.
7. Deng G, Peng E, Gum J, Terdiman J, Slesinger M, Kim YS (2002) Methylation of hMLH1 promoter correlates with the gene silencing with a region-specific manner in colorectal cancer. *Br J Cancer* 86: 574-9.
8. Dodds L, Davis S, Polisson L (1974) A population-based study of lung cancer incidence trends by histological type. *J Natl Cancer Inst* 76: 21-9.
9. Esteller M, Corn PG, Baylin SB, Herman JG (2001) A gene hypermethylation profile of human cancer. *Cancer Res* 61: 3225-9.
10. Esteller M, Levine R, Baylin SB, Ellenson LH, Herman JG (1998) MLH1 promoter hypermethylation is associated with microsatellite instability phenotype in sporadic endometrial carcinomas. *Oncogene* 16: 2413-7.
11. Hansen LT, Thykjaer T, Orntoft TF, Rasmussen LJ, Keller P, Spang-Thomsen M, Edmonston TB, Schmutte C, Fishel R, Petersen LN. (2003) The role of mismatch repair in small-cell lung cancer cells. *Eur J Cancer* 39: 1456-67.
12. Herman JG, Umar A, Polyak K, Graff JR, Ahuja N, Issa JP, Markowitz S, Willson JK, Hamilton SR, Kinzler KW, Kane MF, Kolodner RD, Vogelstein B, Kunkel TA, Baylin SB (1998) Incidence and functional consequences of hMLH1 promoter hypermethylation in colorectal carcinoma. *Proc Natl Acad Sci U S A* 95: 6870-5.
13. Ko YC, Wang JL, Wu CC, Huang WT, Lin MC. (2005) Lung cancer at a medical center in Southern Taiwan. *Chang Gung Med J*. 28(6): 387-95
14. Lam WK, So SY, Yu DYC (1983) Clinical features of bronchogenic carcinoma in Hong Kong: review of 480 patients. *Cancer* 52: 369-76.
15. Leung SY, Yuen ST, Chung LP, Chu KM, Chan ASY, Ho JCI. (1999) hMLH1 promoter methylation and lack of hMLH1 expression in sporadic gastric carcinomas with high-frequency microsatellite instability. *Cancer Res* 59: 159-64.
16. Lienert T, Serke M, Schonfeld N, Loddenkemper R. (2000) Lung cancer in young females. *Eur Respir J* 16: 986-90.
17. Mackay HJ, Cameron D, Rahilly M, Mackean MJ, Paul J, Kaye SB, Brown R. (2000). Reduced MLH1 expression in breast tumors after primary chemotherapy predicts disease-free survival. *J Clin Oncol* 18: 87-93.
18. Minami H, Yoshimura M, Miyamoto Y, Matsuoka H, Tsubota N. (2000) Lung cancer in Women. Sex-associated differences in survival of patients undergoing resection for lung cancer. *Chest* 118: 1603-9.
19. Ryberg D, Hewer A, Phillips DH, Haugen A (1994). Different susceptibility to smoking-induced DNA damage among male and female lung cancer patients. *Cancer Res* 54: 5801-3.
20. Rhyu MS. (1996). Molecular mechanisms underlying hereditary nonpolyposis colorectal carcinoma *J Natl Cancer Inst* 88: 240-51.
21. Stolley PD. (1983) Lung cancer in women: five years later, situation worse. *N Engl J Med* 309: 428-9.
22. Strathdee G, MacKean MJ, Illand M, Brown R (1999) A role for methylation of the hMLH1 promoter in loss of hMLH1 expression and drug resistance in ovarian cancer. *Oncogene* 18: 2335-41.
23. Takagi Y, Koo LC, Osada H, Ueda R, Kyaw K, Ma CC, Suyama M, Saji S, Takahashi T, Tominaga S, et al (1995) Distinct mutational spectrum of the p53 gene in lung cancers from Chinese women in Hong Kong. *Cancer Res* 55: 5354-7.
24. Veigl ML, Kasturi L, Olechnowicz J, Ma AH, Lutterbaugh JD, Periyasamy S, Li GM, Drummond J, Modrich PL, Sedwick WD, Markowitz SD. (1998) Biallelic inactivation of hMLH1 by epigenetic gene silencing, a novel mechanism causing human MSI cancers. *Proc Natl Acad Sci U S A* 95: 8698-702.
25. Wang YC, Chen CY, Chen SK, Cherng SH, Ho WL, Lee H. (1998) High frequency of deletion mutation in p53 gene

from squamous cell lung cancer patients in Taiwan. *Cancer Res* 58: 328-33.

26. Wang YC, Lu YP, Tseng RC, Lin RK, Chang JW, Chen JT, Shih CM, Chen CY. (2003) Inactivation of hMLH1 and hMSH2 by promoter methylation in primary non-small cell lung tumors and matched sputum samples. *J Clin Invest* 111: 887-95.

27. Ward RL, Cheong K, Ku SL, Meagher A, O'Connor T, Hawkins NJ (2003) Adverse prognostic effect of methylation in colorectal cancer is reversed by microsatellite

instability. *J Clin Oncol* 21: 3729-36.

28. Wu AH, Henderson BE, Pike MC, Yu MC. (1985) Smoking and other risk factors for lung cancer in women. *J Natl Cancer Inst* 74: 747-51.

29. Xinarianos G, Liloglou T, Prime W, Maloney P, Callaghan J, Fielding P, Gosney JR, Field JK. (2000) hMLH1 and hMSH2 expression correlates with allelic imbalance on chromosome 3p in non-small cell lung carcinomas. *Cancer Res* 60: 4216-21.

Author Information

Joseph Skarda, M.D., D.V.M.

Department of Pathology, Olomouc LF Palacky University

Eduard Fridman, M.D.

Department of Pathology, The Chaim Sheba Medical Center and Sackler School of Medicine, Tel Aviv University

Pavlina Plevova, M.D.

Department of Pathology, Olomouc LF Palacky University

Marian Hajduch, M.D., Ph.D.

Laboratory of Experimental Medicine, Pediatric Department, Olomouc LF Palacky University

Efrat Ofek, M.D.

Department of Pathology, The Chaim Sheba Medical Center and Sackler School of Medicine, Tel Aviv University

Jury Kopolovic, M.D.

Department of Pathology, The Chaim Sheba Medical Center and Sackler School of Medicine, Tel Aviv University

Zdenek Kolar, M.D., Ph.D.

Department of Pathology, Olomouc LF Palacky University