고신대학교 의과대학 학술지 제23권 제3호
Kosin Medical Journal
Vol. 23. No. 3, pp. 17~21, 2008

Expression of MAGE in Pleural Fluid for the Differential Diagnosis of Benign and Malignant Pleural Effusion.

Chul-Ho Oak, Tae-Won Jang, Seong-Hoon Shin, Mann-Hong Jung

Department of Internal Medicine Kosin University College of Medicine, Busan, Korea

Abstract

Background : Many tumor specific antigens have been studied for tumor diagnosis and immunotherapy. Among tumor specific antigens , Melanoma antigen gene(MAGE) is exclusively expressed in the testis or malignant cells. We investigated MAGE expression in pleural fluid to differentiate malignant from benign pleural effusion. And the results were compared with those of cytologic examination and tumor maker(CEA)

Method : we studied 56 patients with pleural effusions to the Kosin University Gospel Hospital between April 2002 and April 2004(31 men and 25 women: mean age, 56 years). Expression of MAGE was examined by RT-PCR method using a commercial kit. Tumor maker (carcinoembryonic antigen[CEA]) in pleural fluid was determined by immunoassay. Thirty six patients were proven to have malignant pleural effusion by cytology and pleural biopsy, while 20 patients had benign pleural effusion.

Results : MAGE was not expressed in 20 patients with benign pleural effusion, while it was expressed in 23 patients (63.8%) of 36 patients with malignant effusion. The sensitivity of cytologic examinations were 50%. At 100% specificity, a pleural CEA > 50ng/mL had 20% overall sensitivity. The combination of cytology and MAGE reached 84% sensitivity, whereas the combined use of the cytology and tumor marker and MAGE increased sensitivity up to 92%. More than one third of cytology-negative malignant pleural effusion had expression of MAGE.

Conclusion : Expression of MAGE in pleural fluid would be a useful and complementary method for differential diagnosis between benign and malignant pleural effusion.

Key words : MAGE, Cytology, Pleural effusion, Tumor marker, Diagnosis

Introduction

The exact diagnosis of malignant pleural effusions is crucial in many clinical situations. Several diagnostic tests such as cytologic examination, pleural biopsy and thoracoscopy have been utilized to accurately detect malignant pleural effusion. Although repeated large volume thoracentesis and closed needle biopsy increase the yield to 74% for malignant effusion, 20-25% of cases remain undiagnosed.[1] Thoracoscopy will extablish the diagnosis in approximately 95% of cases.[2] But the invasive procedure may not be indicated in all patients nor available at all facilities. Several previous studies demonstrated that many biologic makers including tumor markers, tumor specific antigens are significantly higher in malignant pleural effusion than in benign pleural effusion.

Melanoma antigen gene(MAGE) is one of tumor specific antigens which has been recently studied for tumor diagnosis targets for chemotherapy and immunotherapy.[3,4] MAGE is not expressed in normal tissues except testis and placenta,[5] while it is expressed in various malignant tissues, including malignant melanoma, lung cancer, breast cancer, esophageal cancer, hapatocellular carcinoma, ovarian carcinoma.[6-9] These findings suggest that MAGE may serve as a diagnostic method for detect malignant pleural effusion. To our knowledge, there have been rare previous studies of expression of MAGE in pleural effusion.

The aim of this study was to determine clinical application of MAGE as a predictor of malignant pleural effusions in patients with lung cancer. We also assessed the incremental diagnostic rate of MAGE over the cytologic examination and tumor maker(carcinoembryonic antigen, CEA

교신저자 : 정 만 홍
주소 : 602-702, 부산광역시 서구 암남동 34번지
고신대학교 의과대학 내과학교실
TEL : 051-990-6104
FAX : 051-990-3005
E-mail : oaks70@dreamwiz.com

Patients And Methods

Subjects

Forty six patients with pleural effusion, who were admitted to the Kosin University Gospel Hospital between April 2002 and April 2004, were studied. The clinical features of the patients are shown in Table 1. The study group included 34 men 22 women, with mean age of 61 years. Malignant pleural effusion was present in 36 patients, while benign pleural effusion was present in 20 patients. Of these lung cancer patients, 14 had adenocarcinoma, 10 had squamous cell carcinoma, 9 had small cell carcinoma, and 3 had metastatic carcinoma (Table1). Malignant pleural effusions was diagnosed in 36: 18 patients by cytologic examination of the pleural fluid, 15patients by pleural biopsy, and 3 patients by video-assisted thoracoscopic biopsy.

Table1. Characteristics of patients with pleural effusion

Characteristics	Malignant effusion	Benign effusion
Age(range)	61.3(43-78)	60.7(42-72)
Sex(M/F)	21/15	13/7
Diagnosis	Primary lung cancer(33)	Tb pleurisy(7)
	Squamous cell carcinoma(10)	Parapneumonic effusion(5)
	Adenocarcinoma(14)	Empyema(5)
	Small cell carcinoma(9)	Congestive heart failure(1)
	Breast carcinoma(2)	Nephrotic syndrome(1)
	Esophageal carcinoma(1)	Liver cirrhosis(1)

Diagnostic Criteria of Pleural effusions.

Malignant pleural effusion was defined exudate with malignant cells on cytologic examination of pleural fluid or pleural tissues. Benign effusion was defined no evidence of malignancy on clinical follow-up of least 12months

Expression of MAGE

Pleural fluid samples obtained by thoracentesis were collected in tubes containing conservative-mixed solution (phenol-guanidinium isothiocyanate, Triton X-100), centrifuged at 1,500g. MAGE expression was reported by means of a commercial kit using RT-PCR method (Figure 1). Total cellular mRNA was extracted from cells and RT-PCR and nested PCR were run in 30 and 35 cycles, respectively, with two different types of primers specially designed to detect six subtypes of MAGE DNA simultaneously, including MAGE-1,-2,-3,-4a,-4b, -5a, -5b,-6. (Table 2)

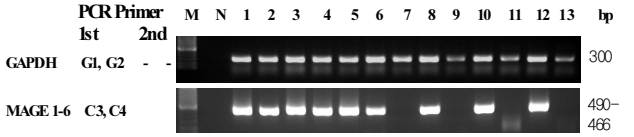

Fig. 1. Amplification of MAGE 1-6 gene in malignant pleural effusion and control group by nested RT-PCR using common primer. G1/G2: sense/antisense primer for GAPDH. M: size marker N: negative control

Table 2. The sequences of multiple MAGEs recognizing primers

Primer	Type	Use	Sequence	SIze
C1	S*	RT-PCR	CTGAAGGAGAAGATCTGCC	828-852
C2	AS**	RT-PCR	CTCCAGGTAGTTTTCCTGCAC	
C3	S*	Nested PCR	CTGAAGGAGAAGATCTGCCW†GTG	466-490
C4	AS**	Nested PCR	CCAGCATTTCTGCCTTTGTGA	

S*, sense primer; AS**, antisense primer
W†, A or T

Tumor Marker Assay

Pleural fluid samples obtained by thoracentesis were collected in tubes containing ethylenediamine tetra-acetic acid, centrifuged at 1,500g, and stored at -70℃ until assayed. Level of CEA was determined using as electrochemiluminescence immunoassay.

Statistical Analysis

Comparison between groups used the χ^2 for categorical variables.

RESULTS

Of the 56 patients who entered the study, 36 patients (16 men and 10 women, with a median age of 61 years) had a malignant pleural effusion, 20 patients (20 men and 24 women, with a median age of 60years) had a benign pleural effusion. The specific etiologies and the histologic subtypes of tumor are presented in Table 1. Among the former group, there were 18 patients with positive pleural fluid cytologic findings. MAGE was not expressed in 20

patients with benign pleural effusion, while it was expressed in 23 patients (63.8%) of 36 patients with malignant effusion. (Table 3). There was no significant difference between detection rates of MAGE and cytologic examination (63% vs 55% respectively; Table 4). There were no statistically significant correlations between expression of MAGE and histologic type of cancer (Table 5).

Table 3. MAGE expression in malignant and benign pleural effusion

	MAGE(+)	MAGE(-)	Total
Malignant effusion	23(63)	13(31)	36
Benign effusion	0	20(100)	20

The number in the parentheses is percent
P < 0.0001

Table 4. Comparision of MAGE and cytologic examination in patients with malignant pleural effusion.

	Cytology(+)	Cytology(-)	Total
MAGE(+)	18(90)	5(31.2)	23(63.8)
MAGE(-)	2(10)	11(69.8)	13(36.2)
Total	20(100)	16(100)	36(100)

The number in the parentheses is percent

Table 5. MAGE expression in malignant pleural effusion according to the tissue types

Tissue type	MAGE(+)	MAGE(-)	Total
Squamous cell carcinoma	6(60.0)	4	10
Adenocarcinoma	9(64.2)	5	14
Small cell carcinoma	6(66.6)	3	9
Metastatic carcinoma	2(66.6)	1	3
Total	23(63.8)	13	36

The number in the parentheses is percent
P < 0.90

Operating Charateristics of Tumor Marker

The median levels of the various tumor markers in the pleural fluid were significantly higher in patients with malignant pleural effusion than in those with benign pleural effusion. (Table 6). The cutoff values of the tumor maker that best differentiated benign from malignant pleural effusion at 100% specificity was 50 ng/ml.

Table 6. Detection rate of malignant pleural effusion by the detection methods

Diagnostic tool	Detection rate (%)
Cytology	55
CEA	20
MAGE	63
Cytology+CEA	70
MAGE + CEA	75
Cytology+MAGE	84
Cytology+CEA+MAGE	92

* the cutoff level for CEA is > 50 ng/ml

Additional Value of MAGE expression over Tumor Marker and Cytology.

The combination of cytology and MAGE reached 84% sensitivity, whereas the combined use of MAGE and tumor marker (CEA) reached 70% sensitivity. Another combination of MAGE plus cytology plus CEA reached up to 92% (Table 6).

Discussion

Pleural involvement is not an uncommon finding in patients with lung cancer. Approximately 10% of lung cancer patients have a pleural effusion at the time of the initial diagnosis, and 30 to 40% develop pleural effusions later in their course.[10] Differentiation between benign and malignant effusion may be important to access the exact staging of the lung cancer for curative intend modality including surgery, concurrent chemoradiation.[11-15] MAGE gene family consists of a large number of chromosome X linked genes originally identified because it encodes products that can be recognized by autologous cytotoxic T cells.[11-15] In search of biomarkers for cancer detection, previous reports have investigated expressions of subtypes of MAGE A from MAGE-1 to MAGE-12 respectively, with most reports showing expression of MAGE genes in only 30-50 % of lung cancer tissues.[16-18] In the study of Park et al., the common primer was designed to detect six subtypes of MAGE DNA simultaneously, including MAGE-1,-2,-3,-4a,-4b, -5a, -5b,-6.[19] In our study, we used the common primer to detect expresson of MAGE in pleural effusion. The higher rate of gene expression found

in this study may be the result of using common primer design that might have improved PCR amplification efficiency. The diagnostic power of PCR was similar to that of cytology. Cytologic examination of pleural effusion has been considered as the simplest method diagnosing malignant pleural effusion. According to one large study, the diagnostic rate by pleural fluid cytology was approximately 62 to 90%.[20] In our study, the diagnostic rate by pleural fluid cytology was 55%. More than one third (31.2%) of cytology-negative malignant pleural effusions had expression of MAGE and the addition of MAGE to the cytologic analysis resulted in a 29% increase of the diagnostic rate, which reflects their complementary value. The role of tumor marker in cancer diagnosis has been unsettled. But many tumor markers have been proposed to aid the diagnostic sensitivity in malignant pleural effusions, including carcinoembryonic antigen, cytokeratin 19 fragment 21-1, carbohydrate antigen 125 and SCC. Among the tumor markers, the sensitivity of CEA (cutoff, 40, 50>ng/mL respectively) in malignant pleural effusion at 100% specificity was reported as 29 to 35%.[21,22] We focused on CEA which is in common use as complementary diagnostic tumor marker. In our study, the sensitivity of CEA (cutoff, 50>ng/mL), which is significantly higher in malignant pleural effusion with 100% specificity was 20%. the combination of MAGE and tumor marker in patients who had negative pleural fluid cytologic examination reached 80% sensitivity.

In conclusion, expression of MAGE in pleural fluid would be a sensitive and specific marker for differential diagnosis between benign and malignant pleural effusion. We recommend the examination of MAGE in patients who have clinical data suggesting malignant pleural effusion, but pleural fluid cytologic examination is negative.

초 록

목 적 : 흉막액을 이용하여 MAGE gene family 중에서 MAGE A1 - A6 유전자를 동시에 검출할 수 있도록 고안된 common primer로 RT-PCR을 시행하여 이러한 검사가 악성 흉막액의 감별에 어느 정도 도움이 될 수 있는지 조사하였다.

대 상 : 2002년 1월부터 2002년 10월까지 고신대학교 복음병원에 흉수를 동반한 질환으로 내원한 환자 중 흉수 세포진 검사나 흉막 조직 검사상 악성 흉막액으로 진단된 환자 36명과 양성 흉막액 환자 20명을 대상으로 하였다.

방 법 : 환자의 흉막액 30mL를 원심 분리하여 상층을 제거한 후 trizol을 이용하여 total RNA를 분리하였다. RNA 분리 후 역전사(reverse transcription)로 cDNA를 합성하여 그 산물로 PCR을 실시하였다. MAGE 1-6 유전자에 대한 RT-PCR & nested PCR-GAPDH로 mRNA가 성공적으로 분리된 것을 확인하였고 MAGE 1-6 common primer (C1,C2/C3,C4)를 사용하여 MAGE 유전자 발현 여부를 확인하였다.

결 과 : 양성 질환에 동반된 흉막액 20예에서는 MAGE가 전례 발현되지 않았고 악성 질환 36예 중 23예 (63.8%)에서 MAGE 가 발현 되었다. ($p<0.0001$). 폐암의 조직형에 따른 MAGE 발현의 차이는 없었다. ($p<0.9$). 악성 질환을 동반한 환자의 흉막액 세포진 검사, CEA 그리고 MAGE 검사의 민감도는 각각 55%, 20%, 63% 였다. 세포진 검사에서 음성이었던 16예 중 MAGE가 11예에서 양성이고 CEA는 7예에서 양성이었다. 흉막액 세포진 검사에 MAGE와 CEA를 추가 조사한 경우는 악성 흉막액의 진단율을 92%로 올렸다.

결 론 : 악성 흉막액의 감별진단에 MAGE의 발현을 조사하는 것이 세포진 검사보다 더 유용하였으며 악성 흉수의 진단에 세포진 검사, 종양 표식자 검사와 함께 MAGE 검사가 보완적인 도구가 될 수 있을 것이다. 향 후 더 많은 환자를 대상으로 한 추가 검사가 필요할 것으로 생각된다.

References

1) Loddenkemper R, Crossler H, Gabler A : Prospective evaluation of biopsy methods in the diagnosis of malignant pleural effusion. Intrapatients comparison between pleural fluid cytology, blind biopsy, and thoracoscopy. Am Rev Respir Dis 127:114-122, 1983
2) Loddenkemper R : Textbook of pleural diseases. London, UK Arnold, 2003, 498-512
3) Itoh K, Hayashi A, Nakoa M, Hoshino T, Seki N, Shichijo SJ : Human tumor rejection antigens MAGE. Biochem 119:385-390, 1996

4) Duan Z, Duan Y, Lamendola DE, Yusuf RZ, Naeem R, Penson RT, Seiden MV. : Overexpression of MAGE/GAGE gene in paclitaxel/doxorubicin resistant human cancer cell lines. Clin Caner Res 9:2778-2785, 2003
5) Jungbluth AA, Busam KJ, Kolb D, Iverson K, Coplan K, Chen YT : Expression of MAGE-antigen in normal tissue and cancer. Int J Cancer 85:460-465, 2000
6) Zambon A, Mandruzzato S, Parenti A, Macino B, Dalerba P, Ruol A : MAGE, BAGE, and GAGE gene expression in patients with esophageal squamous cell carcinoma and adenocarcinoma of the gastric cardia. Cancer 91:1882-1888, 2001
7) Yoshimatsu T, Yoshino I, Ohgami A, Takenoyama M, Hanagiri T, Nomoto K : Expression of the melanoma antigen-encoding gene in human lung cancer. J Surg Oncol 67:126-129, 1998
8) Otte M, Zafrakas M, Riethdorf L, Pichlmeier U, Loning T, Janicke F : MAGE-A gene expression pattern in primary brease cancer. Cancer Res 61:6682-6687, 2001
9) Tahara K, Mori M, Sadanaga N, Sakamoto Y, Kitano S, Makuuchi M : Expression of the MAGE gene family in human hepatocellular carcinoma. Cancer 85:1234-1240, 1999
10) Paas HI, Mitchell JB, Johnson DH : Lung cancer :principles and practice. Philadelphia, Lippincott-Raven, 1996, 643-654
11) van der Bruggen P, Traversari C, Chomez P, Lurquin C, De Plaen E, Van den Eynde B : A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. Science 254:1643-1647, 1991
12) De Plaen E, Arden K, Traversari C, Gaforio JJ, Szikora JP, De Smet C : Genome. Structure chromosomal localization and expression of twelve genes of the MAGE family. Immunogenetics, 40:360-369, 1994
13) Roger UC, Wilke K, Steck E, Korn B, Poustka : The melanoma antigen gene(MAGE) family is clustered in the chromosomal band Xq28. Genomics 29: 725-731, 1995
14) Lucas S, De Plaen E, StaffordAN, Monaco AP : Isolation and characterization of a new MAGE gene family with tumor-specific expression. Int J cancer 87:55-60, 2000
15) Lurquin C, De Smet C, Brasseur F, Muscateli F, Martelange V, De Plaen E : Two members of the human MAGE gene family located in Xp 21.3 are expressed in tumors of various histological origins. Genomics 46:397-408, 1997
16) Jang SJ, Soria JC, Wang L, Hassan KA, Morice RC, Walsh GL, Hong WK : Activation of melanoma antigen tumor antigens occurs early in lung carcinogenesis. Cancer Res 61:7959-7963, 2001
17) Lucas S, De Smet C, ArdenKC, Viars CS, Lethe B, Lurquin C : Identification of a new MAGE gene with tumor-specific expression by representional difference analysis. Caner Res 58:743-752, 1998
18) Weynant P, Lethe B, Brasseur F, Marchand M, Boon T : Expression of MAGE genes by non-small cell lung carcinoma. Int J Cancer 56:826-829, 1994
19) Park JW, Kwon TK, Kim IH, Sohn SS, Kim YS, Kim CT : A new strategy for the diagnosis of MAGE-expressing cancers. Immumol Methods 266:79-86, 2002
20) American Thoracic Society : Management of malignant pleural effusion. Am J Respir Crit Care Med 162:1987-2001, 2000
21) Ferrere F, Villarino MA, Encabo G : Diagnostic utility of CYFRA 21-1, carcinomembryonic antigen, CA 125, neuron specific enolase, and squamous cell antigen level determinations in the serum and pleural fluid of patients with pleural effusions. Cancer 86:1488-1495, 1999
22) Porcel JM, Vives M, Esquerda A : Use of a panel of tumor makers (carcinoembryonic antigen, cancer antigen 125, carbohydrate antigen 15-3, and cytokeratin 19 fragments) in pleural fluid for the differential diagnosis of benign and malignant effusuions. Chest 126:1757-1763, 2004