

두경부암 환자의 객담으로부터 Common MAGE Primer를 이용한 MAGE A1-6 mRNA의 검출의 의의 - 예비 보고

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Significance of MAGE A1-6 mRNA Expression in Sputa of the Head and Neck Cancer Patients - A Preliminary Report

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ABSTRACT

Background and Objectives : We have reported that the expression of MAGE gene is specific to the mRNA level or protein level in head and neck cancer tissues. In this study, we investigated the applicability of MAGE gene to molecular diagnosis of head and neck cancer by detecting MAGE mRNA with common MAGE primers in sputa of patients. **Materials and Method** : Nested RT-PCR with MAGE common primers were designed by authors and were performed to detect MAGE 1, 2, 3, 4a, 4b, 5a, 5b, and 6 (MAGE A1-6) genes in sputa obtained from 22 cancer patients (17 squamous cell carcinoma of laryngopharynx, 2 lung metastasis after surgery for tongue and hypopharyngeal cancer, 2 neck metastasis from primary lung cancer, and 1 case of sarcoma of hypopharynx) and 40 normal persons. **Results** : Of 17 sputa from squamous cell carcinoma patients, expression of MAGE mRNA was positive in 13 (76.5%) cases. MAGE was detected in 72.7% (8/11) of laryngeal cancer, 50% (1/2) of base of tongue cancer, and 100% in hypopharyngeal (2/2) and tonsillar cancer. According to T stage, T1, T2, T3, T4 was positive in 50% (2/4), 100% (5/5), 66.6% (2/3), and 80% (4/5) respectively. In case of primary lung cancer, metastatic lung cancer, and hypopharyngeal sarcoma, all expressed MAGE mRNA. Among 22 cancer patients, 81.8% (18/22) were positive. MAGE expression was detected in 5% (2/40) of the normal control. **Conclusion** : Nested RT-PCR with common MAGE primers was helpful for assessing the presence of cancer cell in the sputa of the respiratory tract. This examination can be used as a tumor marker for the screening of laryngopharyngeal cancers and early detection of recurrent cancers or lung metastasis after treatment. (Korean J Otolaryngol 2003;46:1070-6)

KEY WORDS : MAGE gene · Sputum · Tumor marker · Head and neck.

MAGE (melanoma antigen gene,
MAGE A gene) 가

. MAGE

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(cytotoxic T lymphocyte, CTL)

.¹⁾ MAGE

MAGE A, B, C, D 가 .

²⁾ MAGE A 12 , chromosome Xq28 .³⁾ ,⁴⁻⁶⁾

MAGE A ,⁷⁾ ,⁸⁾ ,⁹⁾ ,¹⁰⁾ ,¹¹⁾ ,¹²⁾ ,¹³⁾ , 2001 4 2002 12

(testis) , ,

MAGE A 17 ,

MAGE A가 2 , 2 ,

1 22

- 40

63.5

가 10 : 1 .

¹⁴⁻¹⁶⁾ 31.5 10 : 1 .

MAGE A 10 : 1 1 : 1 .

Eura ⁴⁾ Lee ⁵⁾ RT - 17

PCR 71~ 4 , 7

75%가 1 MAGE A mRNA 11 , , 2

Lee ⁶⁾ mono- . T T1 4 , T2 5 , T3 3

clonal antibody 57B T4가 5 1

MAGE A가 1 , 3 ,

MAGE A 13 .

mRNA

. Park ¹⁷⁾ mRNA

MAGE A MAGE A mRNA

가 MAGE A1 MAGE A6 1 , , ,

common MAGE primer 1 .

가

nested RT - PCR 70.4% 가

MAGE A가 50 ml .

RNA

(tumor marker) 가 .

50 ml 3~5 ml

(Guanidium thiocyanate, Triton X - 100)

MAGE A mRNA . Magnetic bead(AB-gene, Epsom, U.K.) 150 μ l(7.5 mg)

MAGE A 30 1,100

가 가 . rpm 2 ma-

common MAGE gnetic bead 1.5 ml . 4

primer MAGE A , magnetic bead

가 가 가 70

MAGE A 1400 rpm 10 .

두경부암 환자의 객담에서 MAGE A 유전자의 검출

, biotin oligo(dT)20(10 pmol/
 μ l) 3 μ l 150 μ l
 . SMP(Streptavidin Magnetic Particles, 10
 mg/ml, Roche Diagnostics, Mannheim, Germany)
 , 37 10 . SMP
 3 , SMP
 , 가 3
 20 μ l 가 SMP 70
 mRNA .
 RT - PCR nested PCR
 역전사(Reverse transcription)에 의한 cDNA의 합성
 5X RT buffer 3 μ l, 10 mM dNTP mix 1.5 μ l,
 MMLV reverse transcriptase(200 U/l, Promega, USA)
 0.5 μ l, RNase inhibitor(40 U/l, Promega, USA) 0.5 μ l,
 100 pM oligo dT primer 1.5 μ l PCR tube RT -
 mixture . RT - mixture mRNA 13 μ l
 가 mineral oil 1 10
 . 42 60
 cDNA PCR .

MAGE A1-6 유전자에 대한 C1/C2 primer를 이용한
 일차 PCR

GAPDH(sense : CGTCTTCACCACCATGGAGA, an-
 tisense : CGGCCATCACGCCACAGTTT)

mRNA가 MAGE

A1 - 6 gene PCR . 10X PCR

buffer 3 μ l, 25 mM MgCl₂ 1.8 μ l, 10 mM dATP 0.3

μ l, 10 mM dGTP 0.3 μ l, 10 mM dTTP 0.3 μ l, 10

mM dCTP 0.3 μ l, 50 μ M sense antisense primer

0.25 μ l, Taq polymerase(5 U/ μ l, Promega Co., USA)

0.25 μ l

25 μ l PCR mixture . PCR mixture

PCR tube 5 μ l

mineral oil 1 PCR
 (Cetus 480, Perkin Elmer Co., USA)
 C1/C2 common MAGE primer 1
 PCR (Table 1). 94 5 가
 94 30 , 57 45 , 72 45
 1cycle 35cycle cDNA ,
 72 5 PCR .
 MAGE A1-6 유전자에 대한 C3/C4 primer를 이용한
 nested PCR
 PCR 10 nested PCR
 PCR mixture
 PCR 2 μ l 3 μ l 가 . PCR
 mineral oil 1
 PCR machine C3/C4 primer
 nested PCR (Table 1). 94
 5 가 94 30 , 57 45 ,
 72 45 1 cycle 35 cycle
 cDNA , 72 5
 PCR . 1% agarose gel PCR
 UV transilluminator

MAGE A

χ^2 - test , p<0.05

17 22
 40 MAGE A1 - 6 mRNA
 common primer C1/C2 C3/
 C4 nested RT - PCR

Table 1. Common primer sequences for the detection of MAGE A1-6 genes

Primer	Type	PCR	Sequence	Size (bp)
C1	S	RT-PCR	CTGAAGGAGAAGATCTGCC	831 - 855
C2	AS	RT-PCR	CTCCAGGTAGTTTCTGCAC	
C3	S	Nested PCR	CTGAAGGAGAAGATCTGCCWGTG	469 - 493
C4	AS	Nested PCR	CCAGCATTCTGCCTTTGTGA	

S : sense primer, AS : antisense primer, W : A or T

A1 - 6 mRNA
MAGE A mRNA가 (Fig. 1A).
장기별 발현 빈도
72.7%(8/11), 50%(1/2), (2/2) (2/2) 100%

Table 2. Common primer sequences for the detection of MAGE A1-6 genes

	Disease	% of positive
HN SCC	Larynx	72.7% (8/11)
	Hypopharynx	100 % (2/ 2)
	Base of tongue	50 % (1/ 2)
	Tonsil	100 % (2/ 2)
HN sarcoma	Hypopharynx	100 % (1/ 1)
Lung	Lung cancer with neck metastasis	100 % (2/ 2)
	Lung metastasis of HN cancer	100 % (2/ 2)
Total (n=22)		81.8% (18/22)
HN ; head and neck, SCC ; squamous cell carcinoma		

MAGE A mRNA가(+) (Table 2).
병기별 발현 빈도
1 4 2 (50%), 2 5 5
(100%), 3 3 2 (66.6%), 4 5 4
(80%) MAGE A가 (Table 3).
가

MAGE A1 - 6 mRNA (Fig. 1A)(Table 2).

(2/2), (2/2), (1/1) 100%
MAGE A mRNA가

MAGE A1 - 6 mRNA

1

MAGE A

Table 3. MAGE expression by nested RT-PCR with common primer in squamous cell carcinoma of the head and neck according to T stage

T stage	T1	T2	T3	T4	Total
Primary					
Glottic	50% (1/2)	100% (1/1)	100 % (1/1)	0% (0/0)	75% (3/ 4)
Supraglottic	50% (1/2)	0% (0/0)	50 % (1/2)	100% (3/3)	71% (5/ 7)
Hypopharynx	0% (0/0)	100% (2/2)	0 % (0/0)	0% (0/0)	100% (2/ 2)
Base of tongue	0% (0/0)	0% (0/0)	0 % (0/0)	50% (1/2)	50% (1/ 2)
Tonsil	0% (0/0)	100% (2/2)	0 % (0/0)	0% (0/0)	100% (2/ 2)
Total	50% (2/4)	100% (5/5)	66.6% (2/3)	80% (4/5)	76% (13/17)

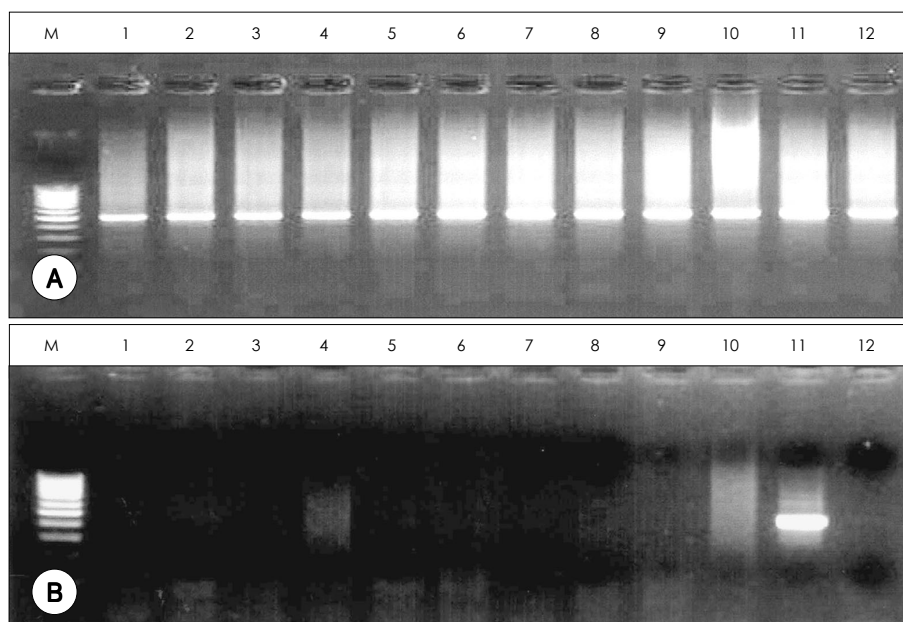


Fig. 1. A : Detection of MAGE mRNA in the sputum of the head and neck cancer. M : size marker. B : Detection of MAGE mRNA in sputum of normal control. Expression of MAGE is noted in Lane 11. Lane 1 - 9 : head and neck cancer, Lane 10 : metastatic lung cancer, Lane 11 : lung cancer with neck metastasis, Lane 12 : sarcoma of the hypopharynx.

두경부암 환자의 객담에서 MAGE A 유전자의 검출

가 11, 6 MAGE A 가 가

MAGE A

6 3 MAGE A 가

2 가

1 MAGE A MAGE A

가 가

MAGE A1 - 6 mRNA 76.5%(13/17)

MAGE A

40 2

(5%) MAGE A가 (p<0.001)(Fig. 1B).

MAGE A가 2

Matsuda ²⁰⁾

Matsuda ²⁰⁾

(sputum cytology)

63.5%, 77.4%

가 , T1 29.4%, T2가

63.3%, T3 69.7%, T4 79.2%

가 ,

가

Matsuda ²⁰⁾ 가

가

가

가

가

T1 50%, T2 100%

MAGE A

가

MAGE A(+)

1 ml 1~4 가

가

¹⁷⁾

Eura ⁴⁾ Lee ⁵⁾⁶⁾ MAGE A 가 MAGE A 가

Lee ¹⁹⁾ Park ¹⁷⁾ MAGE

A1 MAGE A6 nested RT -

PCR common MAGE primer AFP, CEA

5%(2)

MAGE A mRNA

MAGE A가

70.4% MAGE A가 가

PCR, nested RT - RNA, common MAGE primer, MAGE transcripts (early carcinogenesis), Lee⁵⁾, 1, MAGE A mRNA, MAGE A가, 가, : MAGE A, 2001, 1, MAGE A가, 가, MAGE A, 가, MAGE A, 3 (, 2, 1, 가, 1, MAGE A, MAGE A, 가, 2, MAGE A, 가, 가, MAGE A

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