

Differential diagnosis of adenoid cystic carcinoma and pleomorphic adenoma using of MAGE A

Department of Otolaryngology, College of Medicine Chosun University, Gwangju, Korea¹,

Department of Pathology, College of Medicine Chosun University, Gwangju, Korea²

Jun Hee Park M.D.¹, Nam Yong Do M.D.¹, Sung Chul Lim M.D.²

ABSTRACT

This research aims to examine whether the expression pattern of MAGE can be a useful marker for differential diagnosis of pleomorphic adenoma and adenoid cystic carcinoma. The gene expression of MAGE A is observed through Real-time RT-PCR, to confirm its efficiency and legitimacy as a tool of differential diagnosis for pleomorphic adenoma and adenoid cystic carcinoma.

INTRODUCTION

- Pleomorphic adenoma and adenoid cystic carcinoma are starkly different from one another, showing clinically huge differences, their histological diagnoses are extremely similar.
- Many researchers have presented useful markers to distinguish and to diagnose these tumors, however these are not yet being widely used in practice.
- Melanoma antigen gene(MAGE) : antigen-composing coding gene perceived by cytotoxic T lymphocyte from malignant melanomatous cell line.
- Gene expression : expressed in many malignant cells, but not expressed in the normal cells, except in the testicles and the placenta.
- MAGE can play an important role in diagnosis of a tumor.
- Subtype : A, B, C, D

METHODS AND MATERIALS

Immunohistochemical staining

Pleomorphic adenoma : 31 cases

Adenoid cystic carcinoma : 17 cases

Non-neoplastic salivary tissue : 10 cases

- Real-time RT-PCR : PA 3cases,ACA 4cases fresh-frozen at -70°C to be kept

Immunohistochemical stain :

MAGE A mouse monoclonal antibody(Santa Cruz Biotech, U.S.A., 1:200)

MAGE -A4 rabbit polyclonal antibody(ABGENT, San Diego, U.S.A., 1:200)

Real-time RT-PCR

Total RNA was extracted using MagExtractor®, a system of MFX-2100 (Toyobo, Osaka, Japan)

Real-time PCR was carried out through the Light Cycler 2.0 instrument (Roche, Mannheim, Germany) in the use of Tagman Master Mix (Roche)

Judgment of Results of the Immunohistochemical staining

below 1% : negative above 1% : positive

1 ~ 25% : 1+, weak positive 26 ~ 49% : 2+, moderate positive

above 50% : 3+, strong positive

Statistical Analysis

Correlation between the expression of MAGE A and MAGE-A4, and the positive expression rate in the pleomorphic adenoma and adenoid cystic carcinoma were compared.

It is presumed to be statistically significant under the condition of $p < 0.05$

Table 1. Primers, probes and thermal cycling conditions of real-time PCR

Gene	Sense (5' → 3')	Antisense (5' → 3')	Probe (5' → 3')	Annealing-extension
MAGEA1	GCCGAAGGAACCTGACC	ACTGGGTTGCCTCTGTGG	TGTGTGCAGGCTGCCACCTCCT	90 s, 65°C
MAGEA2	AAGTAGGACCCGAGGCACTG	GAAGAGGAAGAAGCGGTCT	CATTGAAGGAGAAGATCTGCCTGTGGTCTTC	1 min, 60°C
MAGEA3	GTCGTGGAAATTGGCAGTA	GCAGGTGGCAAAGATGTAC	AAAGCTTCCAGTTCCTT	1 min, 62°C
MAGEA4	CCACTACCATCAGCTTCACCTT	CTTCTCGGAACAAGGACTCT	AGGCAACCCAATGAGGGTTCCAGC	1 min, 63°C
MAGEA6	GTCGTGGAAATTGGCAGT	GCAGGTGGCAAAGATGTAC	TGCAAGGAATCGGAAGC	1 min, 65°C
MAGEA10	TACTGCACCCTGAGGAGGT	TGTGGTGGCAATTCTGTCT	AAATGGGAGTGATCCAAGATCCTTCCCA	1 min, 64°C
MAGEA12	GGTGAAGTGGTCCGCTCG	GCCCTCCTGATCTTTAGC	AGGCATCTGATGGGAGG	1 min, 60°C
β-actin	GGGAATCTGACGGATCGGA	GGAATGGAACGCCTGGAAC	TGCTCTCGAAGATCGTCATGCCTCC	1 min, 60°C

RESULTS

Table 2. MAGE A expression in pleomorphic adenoma and adenoid cystic carcinoma of the salivary gland.

Tumors	MAGE A expression				Positive (%)
	Negative	Weak	Moderate	Strong	
PA (n=31)	26	5	0	0	5 (16.0)
ACC (n=17)	0	2	7	8	17 (100.0)*

PA : Pleomorphic adenoma
ACC : Adenoid cystic carcinoma
* : Statistically significant $p < 0.05$

Table 3. MAGE-A4 expression in pleomorphic adenoma and adenoid cystic carcinoma of the salivary gland

Tumors	MAGE-A4 expression				Positive (%)
	Negative	Weak	Moderate	Strong	
PA (n=31)	30	1	0	0	1 (3.0)
ACC (n=17)	1	3	9	4	16 (94.0)*

PA : Pleomorphic adenoma
ACC : Adenoid cystic carcinoma
* : Statistically significant $p < 0.05$

RESULTS

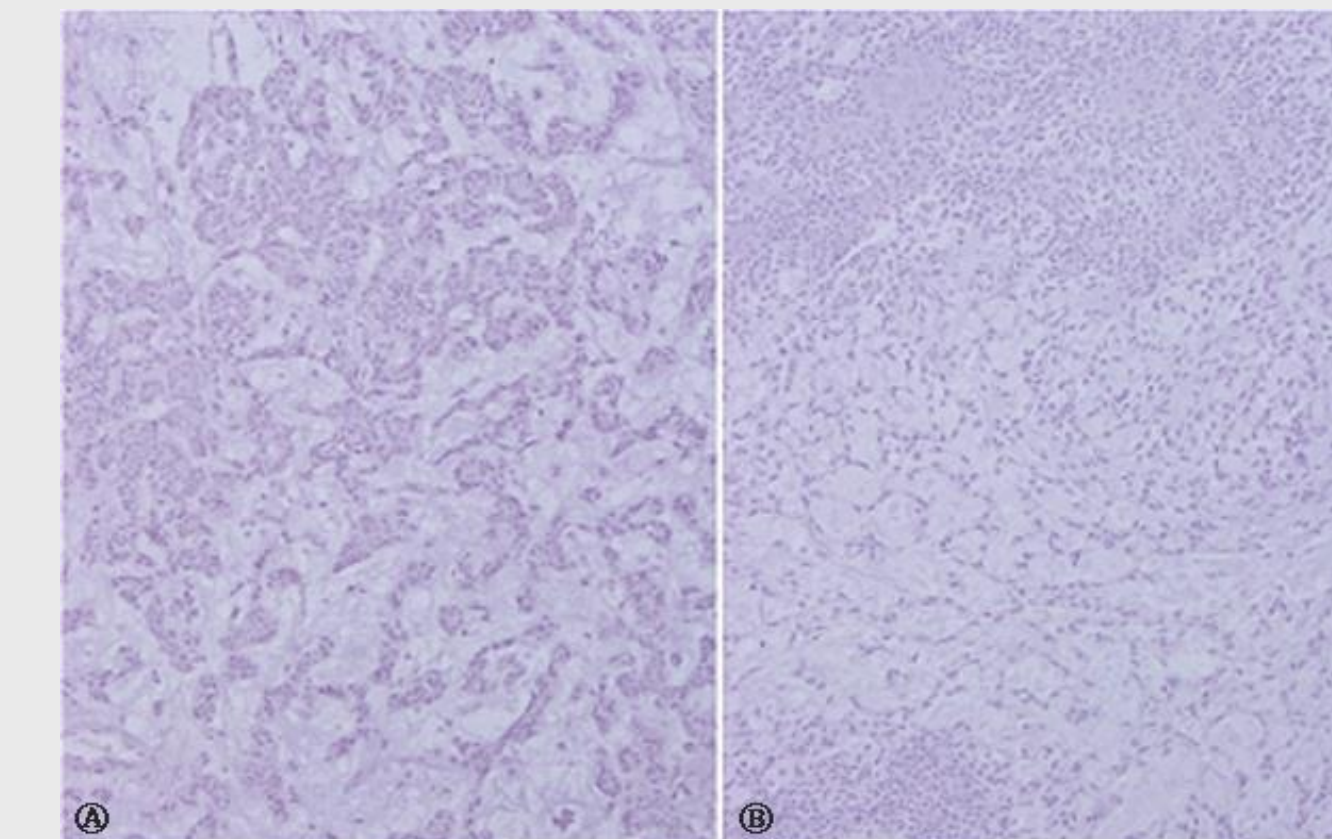


Fig. 1 Immunohistochemical staining of pleomorphic adenoma for MAGE-A. No immunohisto-chemical reaction was identified. Polink-2 HRP plus mouse DAB detection system, counter-stained by hematoxylin. a,b: x100

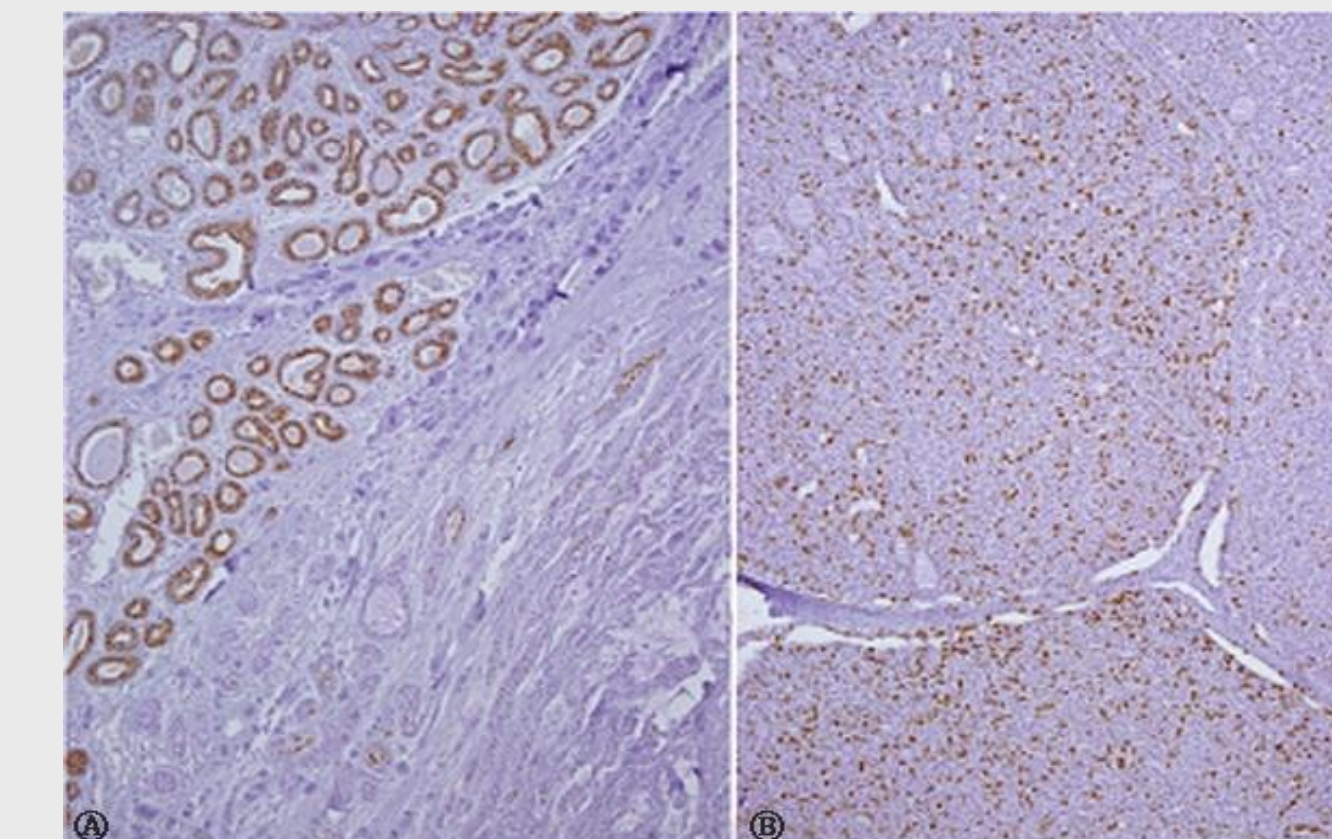


Fig. 3 Immunohistochemical staining of adenoid cystic carcinoma for MAGE-A4. a: Strong positive nuclear staining was identified in tubular structures. b: Diffuse positive immunoreaction was identified in the solid portion of tumor. Polink-2 HRP plus rabbit DAB detection system, counterstained by hematoxylin. a,b: x100

RESULTS

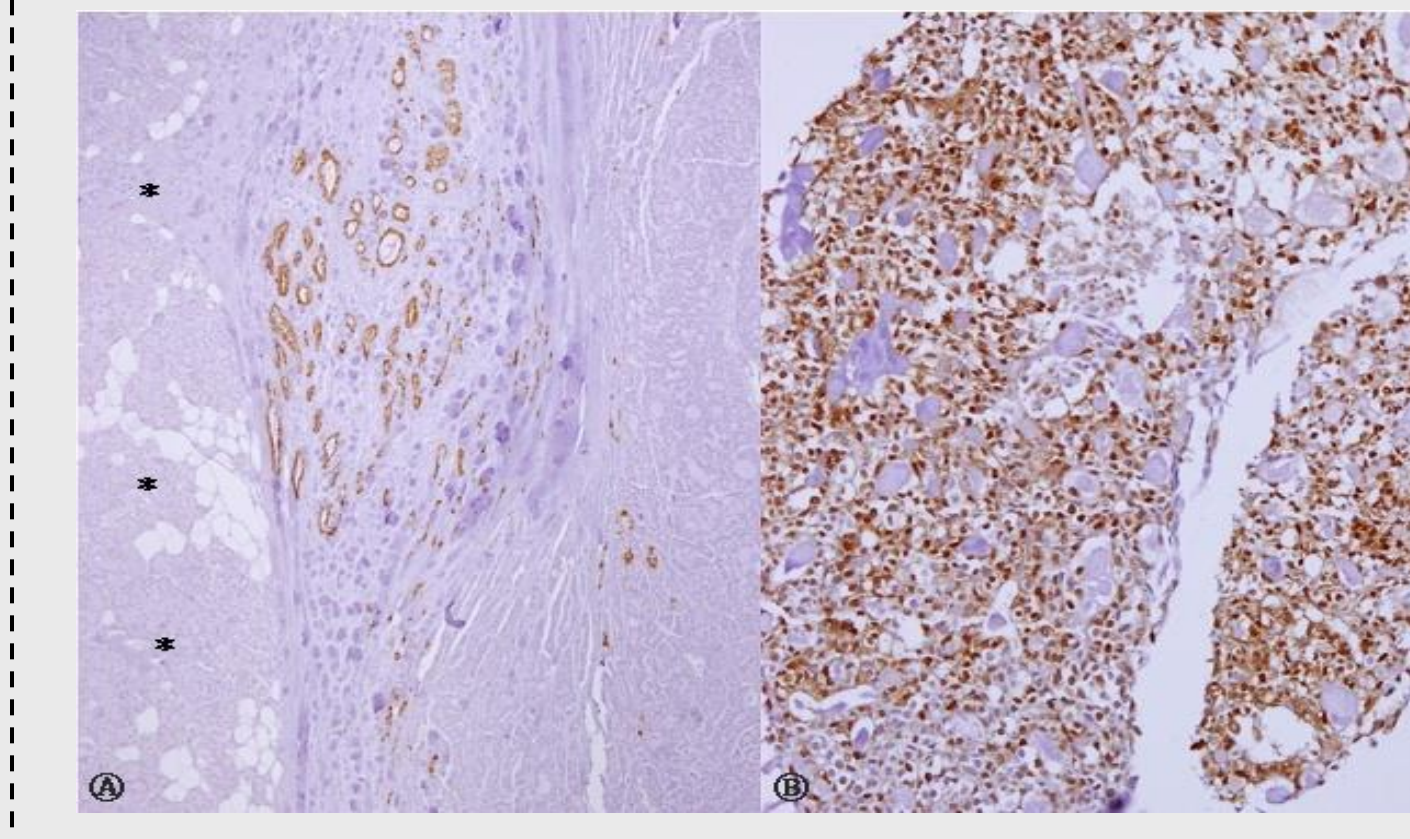


Fig. 2 Immunohistochemical staining of adenoid cystic carcinoma for MAGE-A. a: Strong positive nuclear immunoreaction was identified. Adjacent normal salivary tissue (asterisks) was completely negative for MAGE-A. b: Diffuse positive immunoreaction was demonstrated in tumor cells. Polink-2 HRP plus mouse DAB detection system, counterstained by hematoxylin. a: x40, b: x100

SUMMARY

In 84% of the immunohistochemical stain of MAGE A and PA there was no revelation, but there was revelation in 100% of ACC. In 97% of the immunohistochemical stain of MAGE-A4 and PA there was no revelation, but there was revelation in 100% of ACC.

None of the MAGE gene expression in 3 cases of pleomorphic adenoma tissues were measured. MAGE-A3 and MAGE-A4 were both expressed in 4 cases by using adenoid cystic carcinoma tissue. MAGE-A6 and -A10 were expressed in 2 cases respectively, and MAGE-A12 was expressed in 1 case. Both MAGE-A1 and -A2 were not expressed in pleomorphic adenoma and adenoid cystic carcinoma.

CONCLUSIONS

The characteristics of MAGE were found to show a significant difference in expression at the time of immunohistochemical staining on pleomorphic adenoma and adenoid cystic carcinoma, and this is regarded as a useful marker for overcoming the difficulty in differential diagnosis of the two tumors.

CONTACT

Jun Hee Park M.D.

Department of Otolaryngology, College of Medicine Chosun University, Gwangju, Korea

Email: entjh28@hanmail.net

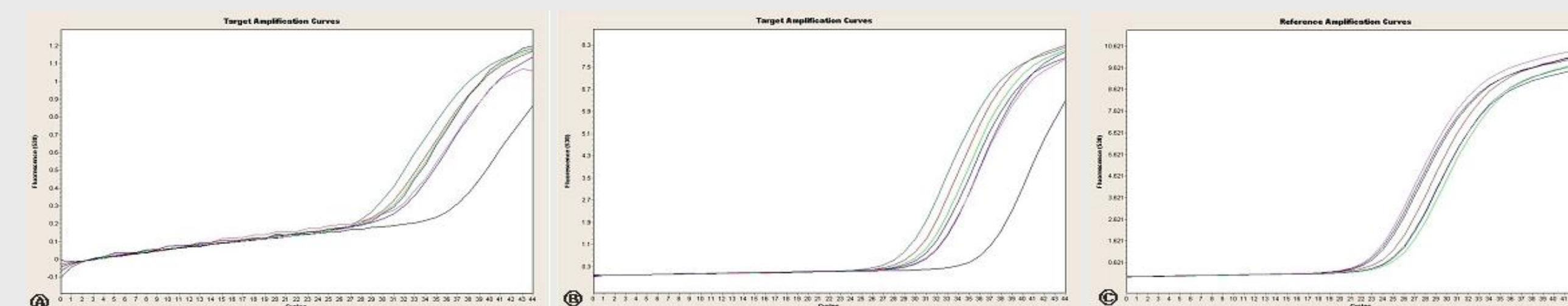


Figure 4. Real-time amplification plot of MAGE. Any MAGE gene was not expressed in PA, but MAGE-A3 and-A4 were expressed in ACC. A: MAGE -A3, B: MAGE-A4, C: Reference Curve

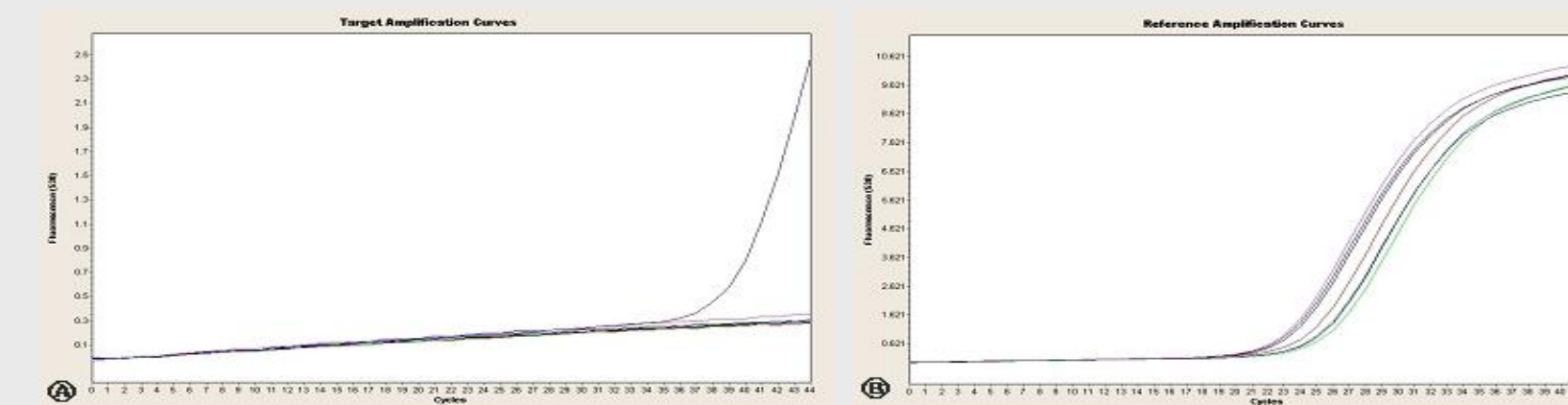


Figure 5. A: Real-time amplification plot of MAGE-A1. The expression of MAGE-A1 gene was not observed in either of PA or ACC. B: Reference Curve.