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SCIENCE DIRECT

Gynecologic Oncology 97 (2005) 342–347

Gynecologic Oncology

www.elsevier.com/locate/ygyno

The expression of MAGE and GAGE genes in uterine cervical carcinoma of Korea by RT-PCR with common primers

Hee-Kyung Chang[a,*], JongWook Park[b], WooGyeong Kim[c], KiTae Kim[c], Minyoung Lee[d], UnDong Park[e], ByungGil Choi[d]

[a]*Department of Pathology, Kosin University Medical College, 34 AmNam-Dong, Suh-Ku, Pusan, 602-702, South Korea*
[b]*Department of Immunology of Keimyung University Medical College, South Korea*
[c]*Department of Obstetrics and Gynecology, Inje University Pusan Baik Hospital, South Korea*
[d]*Institute for Medical Science, Kosin University Medical College, South Korea*
[e]*Department of Gynecology, Kosin University Medical College, South Korea*

Received 3 December 2003
Available online 8 February 2005

Abstract

Background. Melanoma antigen genes (MAGE) and GAGE genes are encoded by genes that are silent in virtually all normal adult tissues but are expressed in tumors from various tissues. These gene products are targets for specific immunotherapy as they are presented by HLA I molecules and recognized by autologous cytotoxic T-lymphocytes. However, the characteristics of these genes, especially in uterine cervical cancer are relatively unknown.

Purpose. This study evaluated the prevalence of MAGE and GAGE by reverse transcription-polymerase chain reaction (RT-PCR) with common primers and discusses clinical implications in cervical carcinoma.

Materials and methods. Fresh tissue from 37 cases of primary squamous cell carcinoma and normal cervical mucosa were evaluated for clinicopathologic parameters including Human Papilloma Virus (HPV)-16,18 infection by PCR, tumor stage by FIGO classification and lymph node involvement. RT-nested PCR for the MAGE and GAGE genes was performed with common primers and DNA sequencing after subcloning was used for identification of PCR products of MAGE. Formalin-fixed paraffin embedded material from the same specimen was analyzed by in situ RT-PCR for MAGE.

Results. Expression of MAGE and GAGE was not observed in normal tissues. Eleven out of 37 cases expressed MAGE mRNA (29.7%): analysis of subtypes identified one case of MAGE-1, two cases of MAGE-4b, six cases of MAGE-3, and two unknown subtypes. Thirteen out of 37 cases (35.1%) expressed GAGE mRNA. No significant relationships between expression of these genes and FIGO staging, lymph node metastasis or HPV infection were found.

Conclusion. Expression of MAGE and GAGE may be involved in the development of uterine cervical carcinoma from intraepithelial neoplasia, although without distinct prognostic significance. MAGE and GAGE genes have the potential to be used as targets for the treatment of uterine cervical carcinoma.

Keywords: MAGE; GAGE; Common primers; HPV-16 and 18; Uterine cervical carcinoma

Introduction

Cancer/testis antigens or tumor-specific shared antigens including MAGE [1,2] and GAGE [3,4] are encoded by genes which are recognized by autologous cytolytic T lymphocytes [1–4]. These antigens may be useful targets for specific immunotherapy, because of their specific expression on tumor cells. Targeted immunotherapy with MAGE products is being tested as a potential new therapy in active cancer [5–9]. MAGE comprises of more than 3 subfamilies: A, B and C. MAGE-A genes represent a family

* Corresponding author. Fax: +82 51 241 7420.
E-mail address: changhkg@ns.kosinmed.or.kr (H.-K. Chang).

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doi:10.1016/j.ygyno.2004.12.051

of more than 12 closely related genes located on the long arm of chromosome X [1,10]. In the majority of tumors investigated, only expression of MAGE-A genes (A1–A4 and A6) has been found [11–15]. The GAGE family of genes includes more than 6 genes [3,4]. The negative association between GAGE gene expression in tumor cells and survival of melanoma patients has been reported recently [16].

Surgical treatment is beneficial for localized early uterine cervical carcinoma. In advanced stages, however, the therapeutic benefit of either the surgical or the chemotherapeutic approach is limited. Therefore, evaluating tumor antigen gene expression in cervical carcinoma is potentially of value for antigen-directed specific immunotherapy. This study determined the prevalence of MAGE and GAGE genes in uterine cervical carcinoma and discusses the clinical implications of using these genes for the design of specific immunotherapy.

Material and methods

Patients and tissue samples

Tissue samples from 37 cases of primary cervical carcinoma and paired normal cervical mucosa were obtained by hysterectomy. All tissue samples were immediately frozen in liquid nitrogen after surgical resection and maintained at −75°C until RNA extraction. The pathologic diagnosis of each tumor confirmed squamous cell carcinoma according to WHO classification. Clinicopathologic parameters including Human Papilloma Virus (HPV)-16,18 infection by PCR, the stage of the tumor by FIGO classification, and lymph node involvement were collected. Of the thirty-seven cervical cancer tissues, four were at stage 0, nine at stage Ia, eighteen at stage Ib and six at stage II. Nine out of 37 had lymph node metastasis (Table 3).

Method

Overview

Total cellular mRNA was extracted, and reverse-transcribed and tested with 35 cycles of reverse transcription-nested polymerase chain reaction (RT-nested PCR) in the presence of primer pairs specific for the β-actin gene and for MAGE and GAGE (Table 1). Duplex PCR of the second round was performed with internal primers for MAGE and GAGE. DNA sequencing after subcloning for identification of PCR products of MAGE was also carried out. Additional material from the same specimen was analyzed by in situ RT-PCR using the same primers as the probes.

Total RNA isolation

Total RNA was isolated using guanidinium isothiocyanate and phenol extraction using a commercial kit according to manufacturer's instructions(RNAzol, Tel-Test, Friendwood, Texas, USA).

cDNA synthesis

cDNA was synthesized from 4 μg of total RNA in a 25-μl reaction mixture containing 6 μl of a 5× reverse transcriptase reaction buffer, oligo(dT) (100 pmol/μl), 4 μl 10 mM dNTP, 40 units/μl RNAsin, 0.5 μl (200 units/μl) Moloney leukemia virus reverse transcriptase (MMLV

Table 1
Oligonucleotide primers for MAGE cDNAs

(A) MAGE family common primers designed for screening by nested PCR

MAGE common primer	Sequence*
1 (S)	CTGAAGGAGAAGATCTGCC
2 (AS)	CTCCAGGTAGTTTTCCTGCAC
3 (S)	CTGAAGGAGAAGATCTGCCWGTG
4 (AS)	CCAGCATTTCTGCCTTTGTGA

(B) Expected sizes of PCR products using the MAGE

Common primer pairs	MAGE Gene (bp)										
	1	2	3	4	4a	4b	5a	5b	6	6G	10
1/2	828	851	852	852	852	852	850	845	851	851	924
3/4	466	489	490	490	490	490	487	487	489	489	562

* Primer pairs 1(sense)/2(antisense) and 3(sense)/4(antisense) are common to MAGE gene 1 through 6 and 10. The locations of sequence 1 and sequence complementary to 2 are as follows; in MAGE 1, bp 473–1,375; MAGE 2, bp 2,842–3,770; MAGE 3, bp 2,307–3,235; MAGE 4a, bp 9,436–10,362; MAGE 4b, bp 2,836–3,762; MAGE 5a, bp 2,922–3,846; MAGE 5b, bp 2,922–3,841; MAGE 6, bp 2,106–3,034; MAGE 10, bp 1,802–2,800. The locations of sequence 3 and sequence complementary to 4 are as follows l in MAGE 1, bp 473–1,013; MAGE 2, bp 2,840–3,408; MAGE 3, bp 2,304–2,873; MAGE 4a, bp 9,436–10,000; MAGE 4b, bp 2,836–3,400; MAGE 5a, bp 2,922–3,483; MAGE 5b, bp 2,922–3,483; MAGE 6, bp 2,103–2,672; MAGE 10, bp 1,799–2,438.