Expression of **BAGE, GAGE, and MAGE** Genes in Human Gastric Carcinoma

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ABSTRACT

The **MAGE, BAGE, and GAGE** genes code for distinct antigens that are recognized by autologous cytolytic T lymphocytes. We investigated the expression of these genes in both cell lines and surgical samples of gastric carcinoma, using reverse transcription-PCR. Furthermore, the induction of these genes by 5-aza-2’-deoxycytidine (DAC), a demethylating agent, was also examined in several cell lines. Of 11 cell lines, **BAGE, GAGEI-6, GAGEI-2, MAGE-1, and MAGE-3** were detected in 7 (64%), 4 (36%), 3 (27%), 8 (73%), and 8 (73%) cell lines, respectively. After the **in vitro** treatment of the negative cell lines with DAC, the expression of these genes became positive in 46 to 91% of these cell lines. No expression of these genes was seen in any of the 57 samples of normal gastric tissue. In contrast, the tumor tissue samples expressed **BAGE, GAGEI-6, GAGEI-2, MAGE-1, and MAGE-3** in 13 (23%), 9 (16%), 6 (11%), 25 (44%), and 23 (40%) tissue samples, respectively. Thus, at least one of these genes was expressed in 35 (61%) of 57 carcinomas. An analysis of the relationship between clinico-pathological factors and the expression of these genes revealed that either **BAGE** or one of these genes was more frequently expressed in histologically intestinal-type than in diffuse-type carcinomas. Our results suggest that, because of the higher expression of these genes and the possible induction of these genes by DAC, patients with gastric carcinoma may, therefore, be potential candidates for tumor-specific immunotherapy directed against these antigens.

INTRODUCTION

A number of genes coding tumor antigens have recently been isolated from human melanoma cell lines. The antigens encoded by these genes are recognized by autologous CTLs in the context of MHC class I molecules, and the antigenic peptides have been identified. Some of these antigens are encoded by genes that are expressed in melanoma and in normal melanocytes (1–5). However, the other antigen genes are expressed in various tumors but are completely silent in normal adult tissue, except for the testis, and these included **MAGE, BAGE, and GAGE** genes (6–8). Furthermore, the tumor antigens caused by point mutations have recently been identified (9, 10). Due to the elicitation of autoimmunity to tumors by these antigens either **in vitro** (11–15) or **in vivo** (16) and their tumor specificity, these antigens thus appear to be potential targets for tumor-specific immunotherapy.

With respect to gastric carcinoma, surgical treatment could be relatively beneficial for localized carcinomas (17, 18). In advanced gastric carcinomas, however, the therapeutic benefit of either a surgical or chemotherapeutic approach seems to still be quite limited (18–20). Therefore, it is considered to be of value to evaluate the therapeutic implications of the tumor antigen gene expression in gastric carcinomas for antigen-directed specific immunotherapy, which is currently being tested in clinical trials for the treatment of melanoma (21).

The **MAGE, BAGE, or GAGE** gene has been shown to be expressed in melanoma and other tumors, including bladder, breast, head and neck, and lung carcinomas (6–8, 22–27). With regard to gastric carcinoma, we demonstrated previously that the expression of **MAGE-1, MAGE-2, and MAGE-3** genes was relatively high compared to that observed in the other tumors (28). In the present study, we examined the expression of **BAGE** and **GAGE**, as well as **MAGE-1** and **MAGE-3** genes, in cell lines derived from human gastric carcinoma, while also investigating the induction of these genes in the cell lines by **in vitro** treatment with DAC, a demethylating agent, since DAC had been shown to up-regulate the expression of the **MAGE-1** gene (29). Furthermore, we also analyzed the expression of these genes in surgical samples of gastric carcinoma and discuss the possibility of potentially using these results for specific immunotherapy in human gastric carcinoma.

MATERIALS AND METHODS

Cell Lines. The cell lines derived from gastric carcinoma, MKN-1, MKN-7, MKN-28, MKN-45, MKN-74, AZ-521, SCH, Ns-8, NUG-C2, NUG-C3, and KATOIII, were supplied by the Japanese Cancer Research Bank (Tokyo, Japan). These cell lines were maintained in RPMI 1640 containing 10% fetal bovine serum and supplemented with 100 units/ml penicillin and 100 mg/ml streptomycin.

Treatment of Cells with DAC. The gastric carcinoma cell lines were grown in medium in flasks in a 5% CO₂ incubator at 37°C. The flasks were wrapped in aluminum foil to protect them from light, and 1 μM DAC (Sigma Chemical Corp., St. Louis, MO) was added for 72 to 96 h. The cells were harvested by trypsinization, and the cell pellets were washed with PBS prior to RNA extraction.

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2 The abbreviation used is: DAC, 5-aza-2’-deoxycytidine.
Patients and Tissue Samples. The tumor tissue samples from primary carcinomas and paired normal gastric mucosal tissues were obtained from resected surgical specimens of patients with gastric carcinoma who had not received chemotherapy. A pathological diagnosis classification of each tumor was performed according to the criteria proposed by the Japanese Research Society for Gastric Cancer (30), including the sex and age of the patient and stage of disease; the depth of tumor invasion; lymphatic permeation; vascular permeation; and pathological diagnosis classification of each tumor was performed according to the criteria proposed by the Japanese Research Society for Gastric Cancer (30), including the sex and age of the patient and stage of disease; the depth of tumor invasion; lymphatic permeation; vascular permeation; and lymph node involvement. All tissue samples were immediately frozen in liquid nitrogen after surgical resection and thereafter were maintained at -70°C until RNA extraction.

PCR Assay for the Expression of BAGE, GAGE, and MAGE genes. The total RNA was isolated using the acid guanidinium thiocyanate-phenol-chloroform extraction procedure (31). cDNA was synthesized from 2.5 μg of total RNA in a 25-μl reaction mixture containing 5 μl of a 5X reverse transcriptase reaction buffer (Life Technologies, Inc., Gaithersburg, MD), 200 μM deoxynucleotide triphosphates (Takara), 500 nM of the appropriate primers, 1 unit AmpliTaq DNA polymerase (PerkinElmer) brought up to volume in 10X PCR buffer (PerkinElmer). The mixture was heated to 94°C for 5 min, and amplification was performed for 30 cycles (1 min at 94°C, 2 min at 62°C, and 2 min at 73°C for BAGE; 1 min at 94°C, 2 min at 56°C (55°C when primer VDE 18 was used), and 3 min at 72°C for MAGE-1 and MAGE-2) or 33 cycles (1 min at 94°C and 3 min at 72°C for MAGE-1; 1 min at 94°C and 4 min at 72°C for MAGE-3). A 10-μl aliquot of each reaction mixture was size-fractionated on 1% agarose gel and visualized with ethidium bromide staining. To ensure that the RNA was not degraded, a PCR assay with primers specific for glyceraldehyde-3-phosphate dehydrogenase cDNA was carried out in each case, except that only 24 cycles were performed, under the following cycling conditions: 1 min at 94°C, 2 min at 56°C, and 2 min at 72°C. The two primer sequences used for the amplification of glyceraldehyde-3-phosphate dehydrogenase mRNA were 5'-GT-CAACGGATTTGCTGATT-3' and 5'-AGTCTTCTGAGTGCA-CGTAGT-3' (33).

Statistical Analysis. The statistical analysis was performed using either the χ² or Fisher’s exact test. The significance level was set at P < 0.05.

RESULTS

Gene Expression and Its Induction by DAC in Cell Lines. Of 11 cell lines derived from gastric carcinoma, the BAGE gene was detected in 7 (64%), whereas the GAGE-1 and GAGE-2 genes were detected in 4 (36%) and 3 (27%) cell lines, respectively (Table 1; Fig. 1). MAGE-1 and MAGE-3 genes were detectable in 8 (73%) cell lines, respectively. After the treatment of the cell lines negative for the expression of these genes with DAC, BAGE, GAGE-1, and GAGE-2 genes were induced in three, four, or two cell lines (Fig. 1), respectively, and MAGE-1 and MAGE-3 genes were also expressed in one cell line, respectively. Therefore, when the negative cell lines were treated with DAC, 10 (91%) of 11 cell lines ex-
pressed BAGE, 8 (73%) expressed GAGE1-6, and 5 (46%) expressed GAGE1-2. In addition, MAGE-1 and MAGE-3 were expressed in 9 (82%) of 11 cell lines, respectively (Table 1).

Expression of Genes in Surgical Specimens. In 57 gastric carcinoma samples studied, BAGE, GAGE1-6, and GAGE1-2 genes were detected in 13 (23%), 9 (16%), and 6 (11%) gastric carcinoma samples, respectively. MAGE-1 and MAGE-3 were expressed in 25 (44%) and 23 (41%) gastric carcinoma samples, respectively (Fig. 2; Table 2). The expression of either MAGE-1 or MAGE-3 was significantly higher than that of BAGE, GAGE1-6, or GAGE1-2. These genes were not detected in any of the matched control samples of normal tissue.

The expression of at least one of BAGE, GAGE1-6, MAGE-1, or MAGE-3 was observed in 35 (61%) of 57 gastric carcinomas. Four (7%) tumors expressed four genes and 10 (18%) tumors expressed three genes. In six members of GAGE gene family (GAGE1-6), only GAGE-1 and GAGE-2 (GAGE1-2) were shown to code for a tumor-specific antigenic peptide presented to CTL (8). The tumor samples that expressed GAGE also expressed one of the BAGE, MAGE-1, or MAGE-3 genes. Thus, the expression of either BAGE, GAGE1-2, MAGE-1, or MAGE-3 was observed in 35 (61%) of 57 tumors.

Gastric carcinomas were separated into groups based on the presence or absence of the expression of these genes, and a comparison of the clinicopathological data was performed (Table 3). The frequency of the expression of either the BAGE gene or at least one of these genes was significantly higher in histologically intestinal-type carcinomas than in diffuse-type carcinomas.

DISCUSSION

The BAGE and GAGE genes, as well as MAGE genes, have been shown to be expressed not only in melanoma but also in
other tumors, including various carcinomas and sarcomas (6–8, 22–27). However, there is still little information on the expression of these genes in gastrointestinal carcinomas. It has been shown that the expression of these genes, MAGE, BAGE, and GAGE, was very low in colorectal carcinomas (7, 8, 23). As shown in our previous study, however, we found that MAGE-1 and MAGE-3 genes were expressed in a relatively high percentage of gastric carcinomas (approximately 40%) in comparison to their expression in the other tumors (28) examined in this study. This report also demonstrated that gastric carcinoma was among the tumors showing the most frequent expression of the BAGE gene (23%), since the highest proportions of positive tumors had been found among melanomas (22%), bladder carcinomas (15%), and breast carcinomas (10%; Ref. 7). With respect to GAGE genes, the incidence of GAGE1-6 expression ranged from 0% in colorectal carcinomas to 30% in melanomas, and the range of GAGE1-2 expression was from 0% in colorectal carcinomas to 25% in sarcomas (8). Therefore, the frequency of the expression of GAGE1-6 (16%) or GAGE1-2 (11%) in gastric carcinomas may be relatively higher than that observed in these tumors. These overall results indicate that gastric carcinomas may exhibit a more frequent expression of these antigen genes, BAGE, GAGE, and MAGE, than other tumors.

The relationship between disease progression and the expression of these genes has been analyzed in melanomas and bladder carcinomas. There was a significantly increased frequency in the expression of MAGE-1, MAGE-2, and MAGE-3 in tumors of greater thickness in melanomas (27). In addition, in bladder carcinomas, MAGE, BAGE, and GAGE genes were more frequently expressed in invasive tumors than in superficial
Table 3  Clinicopathological data on 57 cases of gastric carcinomas

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* Expression at least one of the BAGE, GAGEI-2, GAGEI-6, MAGE-1, or MAGE-3 genes.
* P < 0.05 by Fisher’s exact or χ² tests, as compared to the gene expression in diffuse-type carcinoma.

tumors (7, 8, 26). Furthermore, the frequency of expression of genes MAGE-1, MAGE-3, BAGE, and GAGE was shown to be significantly higher in metastatic lesions than in the primary lesions of melanomas (7, 8, 27). Our data presented above did not exhibit any correlation between the expression of these genes and clinicopathological factors, including the depth of wall invasion, lymph node metastasis, or disease stage in gastric carcinoma. It is noteworthy, however, that the BAGE gene or at least one of these genes was more frequently expressed in histologically intestinal-type carcinomas than in diffuse-type carcinomas. The intestinal type of gastric carcinomas has been shown to clinically exhibit a strong tendency to metastasize to the liver through a hematogenous route as compared to diffuse-type tumors (34, 35). Because the melanoma cells expressing these genes may be capable of metastasizing preferentially, the higher expression of these genes in this subtype of gastric carcinomas may thus be related to the type of biological behavior observed in melanomas.

Because the BAGE and GAGE genes, similar to MAGE genes, were not expressed in normal tissue with the exception of the testis, the antigens encoded by these genes may thus be potentially useful targets for tumor-specific immunotherapy. The MAGE-1 and MAGE-3 genes have been reported to encode for antigenic peptides that were recognized by CTL restricted by HLA-A1 (11, 32), Cw6 (36), or A2 (37). The antigenic peptide encoded by the BAGE gene was shown to be presented by the HLA-Cw6 (7), whereas the GAGEI-2 gene encodes tumor antigen presented by HLA-Cw6 (8). The proportion of HLA-A1 or Cw6 individuals is less than 1% in Japanese (38) but 26% or 16% in Caucasians, whereas that of HLA-Cw1601 individuals is unknown in Japanese, although 7% of Caucasians express this HLA allele (36). However, HLA-A2 is present in 44% of Japanese (38) and in 49% of Caucasians (8). As shown in the expression pattern of BAGE, GAGEI-2, MAGE-1, and MAGE-3 genes, 61% of gastric carcinomas expressed at least one of these genes. After correcting for tumors expressing these genes and for tumors expressing each HLA allele, however, it appears that approximately 20% of Japanese but 36% of Caucasian patients with gastric carcinoma could potentially be eligible for immunotherapy directed against at least one of the presently defined tumor antigens encoded by BAGE, GAGEI-2, MAGE-1, or MAGE-3 genes. In addition, some patients bearing tumors expressing more than one of these antigens could possibly be immunized with a combination of these several antigens, which may thus lead to a potentially more successful outcome.

It was shown that the exposure of MAGE-1-negative melanoma cell lines with DAC, a demethylating agent, in vitro could induce MAGE-1 gene expression; thus, the DAC-treated cells were lysed by a MAGE-1-specific CTL clone (29). The demethylation was considered to subsequently release the MAGE-1 gene from the down-regulatory influences of methylation. Our present results demonstrated that DAC could up-regulate the expression of not only MAGE-1 but also BAGE,
GAGE, or MAGE-3 genes in cell lines derived from human gastric carcinoma. As a result, the majority of gastric carcinoma cell lines, from 46 to 91%, could express these genes, when the negative cell lines were treated with 1 μM of DAC in vitro. The administration of DAC in cancer patients could achieve a serum concentration of from 0.1 to 1 μM with modest toxicity (39). Therefore, the patients with gastric carcinoma exhibiting the negative expression of these antigens may be pretreated with DAC, if the induction can be achieved with an acceptable toxicity, and the patients could thereby be immunized with these antigens.

Due to the relatively high expression of the antigen genes including BAGE, GAGE1-2, MAGE-1, or MAGE-3, many more patients with gastric carcinoma than those with the other tumors could thus be possible candidates for specific immunotherapy directed against these antigens and, in addition, some of these patients could also be eligible for combined immunization with several antigens. Furthermore, it may thus be possible to immunize many more patients with gastric carcinoma against these antigens by pretreatment with DAC. Recently, Marchand et al. (21) reported that 3 melanoma patients of 12 HLA-A1 patients with a tumor expressing MAGE-3 displayed a very significant degree of tumor regression after treatment with the MAGE-3.A1 peptide alone. These results suggest that peptide immunization may thus be effective in a significant proportion of melanoma patients. Our findings, therefore, appear to indicate that immunization with antigens encoded by the BAGE, GAGE1, MAGE-1, or/and MAGE-3 genes, possibly in combination with the pretreatment by DAC, may potentially become a useful modality for the treatment of patients with gastric carcinoma, although MAGE-3 may be a main antigen in the Japanese population based on the prevalence of the gene expression and HLA allelic frequencies. Based on these encouraging findings, further clinical trials are called for.

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