MSI detection. MSI detection was performed as described previously(15). Genomic DNA was extracted from tumor tissues and peripheral lymphcytes. Flagments of microsatellite repeat loci *D2S123*, *D5S346*, *D17S250*, *BAT26*, *BAT25*, *MSH3*, *MSH6*, *TGF β RII*, *BAX*, *MBD4A10*, and *MBD4A6* were amplified PCR, using the above DNA as the template with that from peripheral lymphocytes serving as the normal control. If ≧30 % of the above markers showed MSI, the tumor was defined as MSI-H, based on the standard proposed at the Workshop of the National Cancer Institute Workshop(16). If the MSI rate was less than 30 %, the tumor was defined as MSI-L, and if MSI rate was zero, the tumor was defined as MSS.

Detection of serum CA602 antigen. Serum CA602(17) level was measured by enzyme immunoassay at Mitsubishi BML co. (Tokyo, Japan). Serum CA602 cutoff was set at 63 U/ml based on the values measured in normal individuals (10).

RESULTS

Isolation of cancer testis antigens by SEREX

To isolate cancer testis antigens, we have screened total $1.2x10^6$ clones of a testis cDNA library with the mixture of sera from 3 melanoma patients who were frequently immunized with dendritic cells pulsed with autologous tumor lysates, and an esophageal cancer serum which was known to contain the MAGE cancer testis antigens (as a positive control for isolation of cancer testis antigens). Total 87 positive clones representing 26 distinct genes including cancer testis antigens such as MAGE1a, MAGE2b, MAGE4a, MAGE4b, MAGE6, MAGE9a, and NY-ESO-1, were isolated (Supplemental Table 1). We have also screened a cDNA library made from a mixture of mRNA of 3 endometrial cancer cell lines with sera from 7 patients with endometrial cancer, because endometrial cancer antigens have not yet been isolated by SEREX. Total $5.0x10^6$ cDNA clones were screened, and 193 positive clones representing 59 distinct genes were isolated (Supplemental Table 2). By evaluating tissue specific expression using gene databases including SAGE databases and EST databases, and RT-PCR analysis, one of the isolated clones which showed cancer testis antigen like expression, and identified from both screening of testis and endometrial cancer libraries, was found to be a cancer testis antigen CAGE, which was originally identified by Cho et al. by SEREX with sera from gastric cancer patients(6). They reported that by RT-PCR analysis CAGE was frequently expressed in gastric cancer, cervical cancer, lung cancer, and liver cancer, but the serum recognition was only tested with one gastric cancer patient's serum, which was used for screening the cDNA

library. The expression of CAGE in other cancers including endometrial cancers and melanoma used in this study, and its immunogenicity in patients with various cancers remained to be investigated. Therefore, we attempted further analysis on the CAGE expression and its immunogenicity in various cancers.

Expression of the CAGE mRNA and protein in various cancers including melanoma and endometrial cancers

By RT-PCR analysis, CAGE was expressed in only testis among normal tissues and also in various cancer cell lines including lung cancer, renal cell cancer as previous reported (Fig 1A, B). Although it was previously reported that any of the melanoma, and breast cancer cell lines tested did not express CAGE, we observed that 4 of 7 melanoma, and one breast cancer cell line expressed CAGE. In addition, 2 of 3 endometrial cancer cell lines, one of 3 chronic myelogenous leukemia cell lines and one pancreatic cancer line were found to express CAGE (Fig 1B). We further revealed that 7 of 10 endometrial cancer tissues (4 in grade1, 4 in grade2, and 2 in grade3) and 1 of 3 atypical endometrial hyperplasia tissues expressed CAGE, whereas none of 8 normal endometrium (4 in the proliferation phase and 4 in the secretory phase) did not express CAGE (Fig. 2). The expression of CAGE did not have any correlation with the differentiation grade in endometrial cancer.

Since expression of the CAGE protein in tumor cells has not previously evaluated, we examined the CAGE protein in various cancer cell lines, including endometrial cancer and melanoma, by

Western Blot analysis with the anti-CAGE rabbit antibody which was produced by immunization with the bacterial recombinant CAGE protein as described in the Method section. This polyclonal antibody detected the predicted 82.5kDa band in lysates from NIH-3T3 cells transfected with pcDNA-CAGE, but not in lysates from untransfected NIH-3T3 cells, indicating specific recognition of CAGE (Fig. 3). The same specific bands were shown with this antibody in lysates from 2 endometrial cancer cell lines, Hec-Ib and Ishikawa, and one melanoma cell line 888mel, those are CAGE positive when evaluated by RT-PCR analysis, but were not shown in lysates from PCR negative cultured melanocytes, demonstrating that the CAGE protein was present in various cancer cell lines. Although we further attempted immunohistochemical study with this antibody to confirm the CAGE protein expression in fresh tumor tissues, reliable results could not be obtained by relatively strong background staining with the rabbit serum. We then evaluated subcellular localization of CAGE by immunohistochemical staining of COS cells transfected with pFLAG-CAGE with anti-FLAG M5 murine antibody, and found that the FLAG-CAGE fusion protein was present in nucleus (data not shown) as previously suggested (6).

Detection of anti-CAGE IgG antibody in sera from patients with various cancers including endometrial cancer and melanoma

Since the recognition of CAGE by serum from cancer patients was previously tested only with a single patient with gastric cancer, we have evaluated the recognition of CAGE by serum IgG antibody in patients with various cancers by Western blot analysis with the His-tagged bacterial recombinant

CAGE protein fragment. As a representative Western blot result was shown in Fig.4 and Table1, anti-CAGE IgG antibody was detected in sera from 5 of 45 endometrial cancer, 2 of 24 melanoma, and 2 of 33 colon cancer patients, but not detected in sera from 20 renal cell cancer, 18 prostate cancer, 12 pancreatic cancer patients, or 40 healthy individuals. The positive sera did not stain the negative control protein, recombinant His-tagged VEGFC, assuring the CAGE specific recognition of these positive sera (data not shown). The CAGE specific recognition was also confirmed by phage plaque assay with the same sera (data not shown).

We next attempted quantitative analysis of anti-CAGE IgG antibodies in sera from patients with various cancers using ELISA. By setting up the cutoff for positive anti-CAGE antibody at the average absorbance of the healthy individuals plus 2SD 0.058, positive sera were found in 12 of 45 (26.7%) endometrial cancer, 4 of 33 (12.5%) colon cancer, 2 of 20 (10.0%) melanoma patients, and 1 of 40 (2.5%) age matched healthy individuals, but not in 10 ovarian cancer patients (Fig. 5A). Among these positive patients, 5 endometrial cancer, 2 colon cancer, and 2 melanoma patients demonstrated high CAGE antibody titer over 0.12 OD, for whom positive bands was clearly detected in the Western blot analysis (Fig. 3).

Frequent detection of anti-CAGE serum IgG antibody in patients with MSI positive endometrial cancer

Correlation of the positive serum CAGE antibody with various clinocopathological features was

then evaluated. Although age, FIGO stage, grade, and positive CA602 tumor marker, did not correlate with the positive antibody, surprisingly, the microsattelite instability (MSI) status was found to correlate with the positive antibody in patients with endometrial cancer (p=0.001) (Table 2). Among 33 endometrial cancer patients whose MSI status was evaluated, 7 of 13 (53.8%) patients with MSI-H had positive serum CAGE antibody, while none of 20 non-MSI-H patients including one MSI-L and 19 MSS patients had anti-CAGE antibody (Fig 5). Interestingly, 2 colon cancers with positive CAGE antibody were also turned out to be MSI positive cancers developed in patients with HNPCC (hereditary non-polyposis colon cancer).

Abnormal DNA mismatch repair enzymes in MSI positive tumor cells frequently cause frameshift changes by slippage mutation in the repetitive sequence in the protein coding region. We have previously found that the tumor specific C-terminal CDX2 peptide generated by the frameshift mutation in MSI positive colon cancer induced IgG responses specific for both altered C-terminal peptide and N-terminal wild type peptide in the patient (14). Thus, we have evaluated the possibility that altered CAGE peptide might induce IgG response to CAGE by sequencing the region containing 6 thymine repeat in the CAGE protein coding region of tumors obtained from 5 CAGE antibody positive patients with MSI-H endometrial cancer. However, none was found to have frameshift mutation in this particular repetitive sequence. Therefore, the mechanism for induction of IgG response to CAGE in MSI positive patients has not been clear and needs further investigation.

DISCUSSION

In this study, we attempted isolate cancer testis antigens by screening a testis cDNA library with sera from melanoma patients who were frequently immunized with dendritic cells pulsed with autologous tumor lysates, because mRNA for cancer-testis antigens are often expressed at higher level in testis and cancer cells, and sera from patients immunized with autologous tumor constituents may contain higher titer of antibody specific for immunogenic tumor antigens. We also attempted to isolated endometrial cancer antigens by screening a cDNA library made from endometrial cancer cell lines with sera from patients with endometrial cancer, since SEREX has not previously applied for endometrial cancer. Endometrial cancer is the most common invasive neoplasia of female genital tract and the fourth frequently diagnosed cancer in the United States. Worldwide, approximately 150,000 cases are diagnosed each year, making endometrial cancer the fifth most common cancer in women(18). Since radiation and chemotherapy are not so effective, development of alternative therapeutic strategy such as immunotherapy is required for patients with advanced endometrial cancer.

From these independently performed SEREX studies, a cancer testis antigen CAGE was isolated. CAGE was originally isolated by Cho et al.(6) by SEREX with sera from a gastric cancer patient. CAGE mapped to X chromosome p22.13 was previously shown to express in normal testis and various cancers, including gastric, cervical, lung, and liver cancers. Although function of CAGE has not yet been known, CAGE has helicase domains and DEAD box, and appears to be one of the

DEAD box families with conserved Asp-Glu-Ala-Asp (DEAD) motif, which have RNA-dependent ATPase activity and RNA helicase activity. The DEAD box family proteins are reported to play important roles in a wide range of cellular regulations including RNA metabolism, embryogenesis, spermatogenesis, and cellular growth (19-21). Some DEAD box family proteins, including rck/p54(22), DDX1(23), and HAGE are over-expressed in various cancer cell lines, and the expression of DDX1 are correlated with poor prognosis in patients with neuroblastoma(24). Mutations in helicases involved in DNA repair mechanisms were found in cancer-prone syndromes such as xeroderma pigmentosum, Bloom's syndrome, Werner's disease, X-linked mental retardation associated with α-thalassemia, and Cockayne's syndrome. About the immunogenicity of DEAD box protein, a mutated murine DEAD box protein, named p68, was found to encode an antigens recognized by CTL on a UV-induced sarcoma(25). A mutated peptide of MUM-3 homologous to RNA helicases with a DExH motif, was isolated with HLA-A28 restricted autologous melanoma specific CTL(26).

CAGE was previously reported to express frequently in gastric cancer, cervical cancer, lung cancer, and liver cancer through hypomethylation of promoter region(27). However, expression in other cancers including endometrial cancer and melanoma, has not previously been evaluated. In addition, its immunological recognition has so far been shown only with serum of a single gastric cancer patient. Therefore, we performed further analysis of the protein expression and immunogenicity of CAGE isolated by our 2 independent SEREX experiments using sera from patients with endometrial cancer and melanoma. In addition to the previously reported cancers, we

found that CAGE was also expressed in other types of cancers, including endometrial cancer, melanoma, breast cancer, bladder cancer, pancreatic cancer, renal cell cancer, and leukemia, and in particular it was expressed frequently (7 of 10 patients) in endometrial cancer tissues. Cancer testis antigen frequently expressed in various cancers, MAGE-A4 or NY-ESO-I, was previously reported to be expressed only in 12% or 19% of endometrial cancers, respectively(28). CAGE was also expressed in 1 of 3 atypical endometrial hyperplasia tissues, but not in normal endometrium either proliferation or secretory phase, although cell cycle dependent expression of CAGE was suggested(6). Hypomethylation of the CAGE promoter was reported not only in cancer cells, but also in precancerous states including chronic gastritis and liver cirrhosis, suggesting that CAGE expression may occur in relatively early stage of cancer development.

We next examined immunogenicity of CAGE in patients with various cancers, and found that anti-CAGE IgG antibody were present in sera of patients with various cancers, including endometrial cancers, melanoma, and colon cancer. Since we have been working on immune responses in patients with MSI positive cancers, and subpopulation of endometrial cancer and colon cancer is known to be MSI positive through either mutation of DNA mismatch repair enzyme genes such as MLH1 or silencing of promoters for the repair enzyme genes by methylation, we have evaluated correlation between CAGE antibody positive and MSI status. Particularly, endometrial cancer was reported to be frequently MSI positive due to HNPCC or silencing of the MLH1 promoter by methylation(29). Nine to thirty percent of sporadic endometrial cancers were reported to be MSI positive. Surprisingly, anti-CAGE antibody was detected in sera from 7 of 13 (53.8%) patients with MSI-H, but not in sera

from 20 non MSI-H patients including one MSI-L and 19 MSS patients. Interestingly, 2 colon cancer patients with positive CAGE antibody had also MSI positive cancers developed in patients with HNPCC (hereditary non-polyposis colon cancer). Two melanoma patients with positive CAGE antibody may suggest possible MSI in melanoma, although not evaluated because of unavailability of tumor samples.

Defective DNA mismatch repair frequently causes frameshift changed, unique C-terminal peptides particularly by slippage mutation in the repetitive sequence in the protein coding region. We have previously reported that the CDX2 C-terminal peptide generated by the frameshift mutation induced IgG responses specific for both altered C-terminal peptide and N-terminal wild type peptide in a HNPCC patient(14). Anti-p53 antibody which recognizes wild type p53 was known to be induced through conformational changes of mutated p53. Since CAGE has 6 repeated thymine sequence in the protein coding region, we sequenced this region of genomic DNA obtained from tumor samples of 5 MSI-H endometrial cancer patients, but could not find any alteration in this region. Thus, the mechanism of induction of IgG response to CAGE in MSI positive patients is still unclear. Mutations in other regions of CAGE or other molecules generated by MSI may be involved in modification of the antigen processing and induction of T cells and B cells specific for CAGE.

Since cancer testis antigens are often expressed in HLA negative cells in immunological priviledge sites such as spermatogonia and spermatocytes in tests, they are not recognized by specific T cells, indicating some of the cancer testis antigens may be tumor specific common antigens and one

of the promising targets for cancer immunotherapy. Immunization trials have been in progress for MAGE and NY-ESO-1(30). The recognition by IgG Ab suggests that the same antigen activated CD4+ helper T cells in the patients, meaning that the antigens are immunogenic in cancer patients. In addition, many SEREX defined antigens including MAGE and NY-ESO-1, have been shown to also induce CD8+ cytotoxic T cells (CTL). Positive correlation was observed between positive serum IgG antibody and induction of CD8+ CTL against a cancer-testis antigen NY-ESO-1(31). Patients with MSI positive colon cancer have relatively good prognosis despite of poor histology. Since predominant infiltration of T cells, particularly CD8+ T cells, is observed in MSI positive colon cancer tissues, immune responses to frameshift antigens may contribute to the maintenance of tumor free status after treatment. We have previously demonstrated the immune response to both frameshift mutated and wild type peptides of CDX2 in MSI positive colon cancer patients(14), and T cell response to the frameshift mutated TGFβ-RII frequently detected in MSI positive colon cancer was also reported(32). Although prognosis of MSI positive endometrial cancer is still controversial, there are reports showing better prognosis of patients with MSI positive endometrial cancer(33). If immune response is involved in the good prognosis, CAGE may be one of the target antigens besides the frameshift antigens. Therefore, CAGE may be a good candidate antigen for immunotherapy, at least as CD4+ T cell antigens, particularly for MSI positive endometrial cancer patients with positive CAGE serum antibody.

Serum anti-CAGE antibody may be utilized as a tumor marker. We often observed disappearance of serum antibody to the SEREX defined antigens after curative treatment in patients

with various cancers(13, 14). Use of serum antibodies against p53(34), Cyclin B1(35), hTERT(36, 37) and survivin(37), were recently reported. Positive rate of 15% for anti-p53 antibody in colon cancers and that of 21.6% or 7.8% for anti-survivin antibody in lung or in colon cancer patients were reported. Positive rate of anti-CAGE antibody in 7 of 13 (53.8%) of MSI positive endometrial cancer patients and 1 of 3 of ATH patients indicated possible use of anti-CAGE serum antibody for prognostic or early diagnosis for patients with MSI positive endometrial cancers. Further analysis with a larger numbers of patients is necessary for confirmation and usefulness of this possibility. CA602, a part of CA125 antigen, is one of the most commonly used tumor markers for endometrial cancers. No correlation was observed between anti-CAGE antibody and CA602 in this study. Although CA602 produced by tumor cells correlates with tumor volume, the induction of antibody was defined by immune response of patients through antigen processing and immune response of T cells and B cells. Therefore, these tumor markers can be independently utilized for diagnosis of endometrial cancer.

In summary, we have demonstrated that CAGE is expressed in various cancers including endometrial cancers and melanoma, and frequent detection of specific serum IgG antibody in patients with MSI-H endometrial cancers, indicating that highly immunogenic nature of CAGE in MSI positive endometrial cancers. These results suggest that CAGE may be useful not only for immunotherapy of various cancers, but also for diagnosis of some cancers, particularly MSI positive endometrial cancers.

REFERENCES

1. Kawakami, Y., Fujita, T., Matsuzaki, Y., et al. Identification of human tumor antigens and its implication for diagnosis and treatment of cancer. Cancer Science in press, 2004.
2. van der Bruggen, P., Traversari, C., Chomez, P., et al. A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. Science, *254:* 1643-1647, 1991.
3. Chen, Y. T., Scanlan, M. J., Sahin, U., et al. A testicular antigen aberrantly expressed in human cancers detected by autologous antibody screening. Proc Natl Acad Sci U S A, *94:* 1914-1918, 1997.
4. Scanlan, M. J., Gordon, C. M., Williamson, B., et al. Identification of cancer/testis genes by database mining and mRNA expression analysis. Int J Cancer, *98:* 485-492, 2002.
5. Lucas, S., De Smet, C., Arden, K. C., et al. Identification of a new MAGE gene with tumor-specific expression by representational difference analysis. Cancer Res, *58:* 743-752, 1998.
6. Cho, B., Lim, Y., Lee, D. Y., et al. Identification and characterization of a novel cancer/testis antigen gene CAGE. Biochem Biophys Res Commun, *292:* 715-726, 2002.
7. Nagayama, H., Sato, K., Morishita, M., et al. Results of a phase I clinical study using autologous tumour lysate-pulsed monocyte-derived mature dendritic cell vaccinations for stage IV malignant melanoma patients combined with low dose interleukin-2. Melanoma Res, *13:* 521-530, 2003.

8. Nozawa, S., Sakayori, M., Ohta, K., et al. A monoclonal antibody (MSN-1) against a newly established uterine endometrial cancer cell line (SNG-II) and its application to immunohistochemistry and flow cytometry. Am J Obstet Gynecol, *161:* 1079-1086, 1989.

9. Nozawa, S., Tsukazaki, K., Sakayori, M., Jeng, C. H., and Iizuka, R. Establishment of a human ovarian clear cell carcinoma cell line (RMG-I) and its single cell cloning--with special reference to the stem cell of the tumor. Hum Cell, *1:* 426-435, 1988.

10. Nozawa, S., Udagawa, Y., Sasaki, H., et al. [Studies of clinical usefulness of new tumor markers of ovarian cancer, CA 54/61 and CA 602--CA 602 assay reagent kit, performance its normal value and correlations with other tumor markers]. Gan To Kagaku Ryoho, *19:* 2085-2093, 1992.

11. Kurarmoto, H., Hamano, M., and Imai, M. HEC-1 cells. Hum Cell, *15:* 81-95, 2002.

12. Nishida, M., Kasahara, K., Kaneko, M., Iwasaki, H., and Hayashi, K. [Establishment of a new human endometrial adenocarcinoma cell line, Ishikawa cells, containing estrogen and progesterone receptors]. Nippon Sanka Fujinka Gakkai Zasshi, *37:* 1103-1111, 1985.

13. Kiniwa, Y., Fujita, T., Akada, M., et al. Tumor antigens isolated from a patient with vitiligo and T-cell-infiltrated melanoma. Cancer Res, *61:* 7900-7907, 2001.

14. Ishikawa, T., Fujita, T., Suzuki, Y., et al. Tumor-specific immunological recognition of frameshift-mutated peptides in colon cancer with microsatellite instability. Cancer Res, *63:* 5564-5572, 2003.

15. Hirasawa, A., Aoki, D., Inoue, J., et al. Unfavorable prognostic factors associated with high frequency of microsatellite instability and comparative genomic hybridization analysis in

endometrial cancer. Clin Cancer Res, *9:* 5675-5682, 2003.

16. Boland, C. R., Thibodeau, S. N., Hamilton, S. R., et al. A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res, *58:* 5248-5257, 1998.

17. Nozawa, S., Yajima, M., Sasaki, H., et al. A new CA125-like antigen (CA602) recognized by two monoclonal antibodies against a newly established ovarian clear cell carcinoma cell line (RMG-II). Jpn J Cancer Res, *82:* 854-861, 1991.

18. Parazzini, F., La Vecchia, C., Bocciolone, L., and Franceschi, S. The epidemiology of endometrial cancer. Gynecol Oncol, *41:* 1-16, 1991.

19. Zhao, J., Jin, S. B., Bjorkroth, B., Wieslander, L., and Daneholt, B. The mRNA export factor Dbp5 is associated with Balbiani ring mRNP from gene to cytoplasm. Embo J, *21:* 1177-1187, 2002.

20. Causevic, M., Hislop, R. G., Kernohan, N. M., et al. Overexpression and poly-ubiquitylation of the DEAD-box RNA helicase p68 in colorectal tumours. Oncogene, *20:* 7734-7743, 2001.

21. Tsai-Morris, C. H., Sheng, Y., Lee, E., Lei, K. J., and Dufau, M. L. Gonadotropin-regulated testicular RNA helicase (GRTH/Ddx25) is essential for spermatid development and completion of spermatogenesis. Proc Natl Acad Sci U S A, *101:* 6373-6378, 2004.

22. Akao, Y., Marukawa, O., Morikawa, H., et al. The rck/p54 candidate proto-oncogene product is a 54-kilodalton D-E-A-D box protein differentially expressed in human and mouse tissues. Cancer Res, *55:* 3444-3449, 1995.

23. Godbout, R. and Squire, J. Amplification of a DEAD box protein gene in retinoblastoma cell lines. Proc Natl Acad Sci U S A, *90:* 7578-7582, 1993.

24. Scott, D., Elsden, J., Pearson, A., and Lunec, J. Genes co-amplified with MYCN in neuroblastoma: silent passengers or co-determinants of phenotype? Cancer Lett, *197:* 81-86, 2003.

25. Dubey, P., Hendrickson, R. C., Meredith, S. C., et al. The immunodominant antigen of an ultraviolet-induced regressor tumor is generated by a somatic point mutation in the DEAD box helicase p68. J Exp Med, *185:* 695-705, 1997.

26. Baurain, J. F., Colau, D., van Baren, N., et al. High frequency of autologous anti-melanoma CTL directed against an antigen generated by a point mutation in a new helicase gene. J Immunol, *164:* 6057-6066, 2000.

27. Cho, B., Lee, H., Jeong, S., et al. Promoter hypomethylation of a novel cancer/testis antigen gene CAGE is correlated with its aberrant expression and is seen in premalignant stage of gastric carcinoma. Biochem Biophys Res Commun, *307:* 52-63, 2003.

28. Resnick, M. B., Sabo, E., Kondratev, S., et al. Cancer-testis antigen expression in uterine malignancies with an emphasis on carcinosarcomas and papillary serous carcinomas. Int J Cancer, *101:* 190-195, 2002.

29. Salvesen, H. B., MacDonald, N., Ryan, A., et al. Methylation of hMLH1 in a population-based series of endometrial carcinomas. Clin Cancer Res, *6:* 3607-3613, 2000.

30. Scanlan, M. J., Gure, A. O., Jungbluth, A. A., Old, L. J., and Chen, Y. T. Cancer/testis antigens: an expanding family of targets for cancer immunotherapy. Immunol Rev, *188:* 22-32,

2002.

31. Jager, E., Jager, D., and Knuth, A. Strategies for the development of vaccines to treat breast cancer. Recent Results Cancer Res, *152:* 94-102, 1998.

32. Saeterdal, I., Gjertsen, M. K., Straten, P., Eriksen, J. A., and Gaudernack, G. A TGF betaRII frameshift-mutation-derived CTL epitope recognised by HLA-A2-restricted CD8+ T cells. Cancer Immunol Immunother, *50:* 469-476, 2001.

33. Maxwell, G. L., Risinger, J. I., Alvarez, A. A., Barrett, J. C., and Berchuck, A. Favorable survival associated with microsatellite instability in endometrioid endometrial cancers. Obstet Gynecol, *97:* 417-422, 2001.

34. Winter, S. F., Minna, J. D., Johnson, B. E., et al. Development of antibodies against p53 in lung cancer patients appears to be dependent on the type of p53 mutation. Cancer Res, *52:* 4168-4174, 1992.

35. Covini, G., Chan, E. K., Nishioka, M., et al. Immune response to cyclin B1 in hepatocellular carcinoma. Hepatology, *25:* 75-80, 1997.

36. Masutomi, K., Kaneko, S., Yasukawa, M., et al. Identification of serum anti-human telomerase reverse transcriptase (hTERT) auto-antibodies during progression to hepatocellular carcinoma. Oncogene, *21:* 5946-5950, 2002.

37. Rohayem, J., Diestelkoetter, P., Weigle, B., et al. Antibody response to the tumor-associated inhibitor of apoptosis protein survivin in cancer patients. Cancer Res, *60:* 1815-1817, 2000.

Table 1 Anti-CAGE antibodies was detected in various cancer patients sera by Western blotting analysis

Healthy controls	Endometrial cancer	Ovarian cancer	Melanoma	Colon cancer	Renal cell cancer	Prostate cancer	Pancreas cancer
0/40	5/45	0/10	2/24	2/33	0/20	0/18	0/12

Table 2 The correlation of anti-CAGE antibodies and clinicopathological features

			Anti-CAGE Abs (OD 450nm)		
		n	≦0.058	>0.058	P value[a]
Age					
	≦60	28	20	8	
	>60	17	13	4	0.98
FIGOstage					
	I+II	32	24	8	
	III+IV	13	9	4	0.98
Grade					
	G1+G2	35	28	7	
	G3	10	5	5	0.14
MSI					
	MSI-H	13	6	7	
	MSL+MSS	20	20	0	0.001
	not examined	12			
CA602 value					
	≦63	31	24	7	
	>63	14	9	5	0.58

[a] P value was calculated by χ^2 test.

Figure legends

Fig. 1. Expression of CAGE in various cancers and normal testis evaluated by RT-PCR analysis.

(A) CAGE was expressed only in testis among normal tissues.

(B) CAGE was expressed in 4 of 7 melanoma, 1 of 3 lung cancer, 2 of 4 renal cell cancer, 2 of 3 endometrial cancer, one pancreatic cancer, one bladder cancer, one breast cancer, and 1 of 3 chronic myelogenous leukemia cell lines.

Fig. 2. Expression of CAGE in endometrial cancer tissues, but not in normal endometrial tissues

(A) CAGE was expressed in none of 8 normal endometrium tissues (4 in the proliferation phase and 4 in the secretory phase).

(B) 7 of 10 endometrial cancer tissues (4 in G1, 4 in G2, and 2 in G3) and 1 of 3 AEH tissues expressed CAGE. G1, G2 and G3 are the differentiation grades, grade1, grade2, and grade3, respectively. NC ; negative control, PC : positive control.

Fig. 3. Expression of the CAGE protein in various cancer cell lines