A Novel Cancer Testis Antigen That Is Frequently Expressed in Pancreatic, Lung, and Endometrial Cancers

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Abstract

Purpose: To isolate cancer testis antigens that are expressed in pancreatic cancers and may be useful in clinical applications.

Experimental Design: To efficiently isolate cancer testis antigens, a testis cDNA library was immunoscreened (SEREX) with serum from a patient with pancreatic ductal adenocarcinoma. The expression of isolated antigens in various cancer cell lines and tissues was evaluated by reverse transcription-PCR and Northern blot analyses. The immunogenicity of the antigen in cancer patients was evaluated by detection of the IgG antibody in sera from patients with various cancers.

Results: Of the three clones isolated through screening of a total of 2 × 106 cDNA library clones, one clone (KU-CT-1) was found to be expressed in various cancers but only in testis among normal tissues, indicating that it was a novel cancer testis antigen. The KU-CT-1 gene is located on chromosome 10p12 and produces two splice variants, which encode proteins of 397 and 872 amino acids, respectively. KU-CT-1 was expressed in pancreatic cancer tissues (3 of 9, 33%), lung cancer tissues (9 of 24, 38%), and endometrial cancer tissues (7 of 11, 64%). Specific serum IgG antibodies were detected in 3 of 20 pancreatic cancer patients, 2 of 12 endometrial cancer patients, 1 of 18 colon cancer patients, and 1 of 10 prostate cancer patients but not detected in 30 healthy individuals.

Conclusions: KU-CT-1 is a new cancer testis antigen that is expressed in pancreatic, lung, and endometrial cancers and may be useful for diagnosis and immunotherapy for patients with various cancers.

Pancreatic cancer is a particularly problematic cancer, because early diagnosis is difficult and the tumor is relatively resistant to surgery, radiotherapy, and chemotherapy. Therefore, new diagnostic and therapeutic methods are needed for this cancer. Immunotherapy may be a potential treatment, because various immunotherapies seem to improve the prognosis of patients with pancreatic cancer (1). For the development and improvement of immunotherapy, identification of human tumor antigens is important for evaluation of antitumor immune responses, mechanisms of tumor escape, and more effective immunization (2). However, only a few pancreatic cancer tumor antigens have been identified, including MUC-1 (3), K-ras (4), HER-2/neu (5), and p53 (6).

A variety of human tumor antigens have been identified to date, particularly in melanoma. These include tumor-specific antigens derived from genetic alterations in tumor cells, such as β-catenin and CDK4; tissue-specific proteins, such as PSA, gp100, and proteinase 3; cancer testis antigens, such as MAGE and NY-ESO-1; and antigens that are overexpressed in tumor cells, such as WT1, HER-2/neu, survivin, and hTERT. In pancreatic cancers, recognition of mutated K-ras, HER-2/neu, p53, and MUC-1 by either CD8+ or CD4+ CTLs has been reported (1, 5, 7).

Cancer testis antigens that are expressed in various cancers and normal germ line tissues, such as testis, placenta, and ovary, are a promising class of tumor antigens due to their limited expression in germ line tissues. They are often expressed in MHC class I–negative cells, such as spermatogonia and spermatocytes (8). However, most cancer testis antigens identified to date are expressed relatively rarely in pancreatic cancer (9–14), although SSX-4, SCP-1, and GAGE are reported

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to be expressed relatively frequently in pancreatic cancer tissues. Cancer testis antigens have previously been isolated by various methods, including cDNA expression cloning with tumor-reactive CTLs, patients' sera (SEREX), or cDNA subtractions (15). Among these methods, SEREX seems to be effective for the isolation of cancer testis antigens, and various cancer testis antigens, including MAGEs, SSX2, SCP-1, and CAGE, have been isolated either using cDNA libraries made from tumor cells or testis tissues.

In this study, we attempted to isolate additional cancer testis antigens, which are expressed in pancreatic cancers and are immunogenic in patients with pancreatic cancer, using the SEREX method with a testis cDNA library and serum from a patient with pancreatic ductal adenocarcinoma. One of the isolated novel cancer testis antigens (KU-CT-1) was found to express only in the testis among normal tissues but was expressed frequently in various cancer tissues and, particularly, in pancreatic, lung and endometrial cancers. Furthermore, the IgG antibody specific for this antigen was detected in sera from patients with various cancers, including pancreatic cancer, but not in sera from healthy individuals. Thus, KU-CT-1 may be useful diagnostically and in immunotherapy for patients with various cancers.

### Materials and Methods

**Cell lines and tissues.** The pancreatic duct adenocancer cell lines MiaPaCa2, ASPC1 (from the American Type Culture Collection, Manassas, VA), Panc-1, PK1, PK8, PK9, PK4sp, and PK9 (Tohoku University, Sendai, Japan); the colon cancer cell lines Colo201, Colo320, SW837, RC6, WiDr, DLD-1, and LOVO (JCRB, Osaka, Japan); the stomach cancer cell lines MKN1, MKN7, MKN28, MKN46, and MKN74 (JCRB); the esophageal cancer cell lines TE1, TE2, TE3, TE4, TE5, TE6, TE7, TE8, TE9, TE10, TE11, TE12, TE13, TE14, and TE15 (Tohoku University); the endometrial cancer cell lines Ishikawa, SNGII, EM-tn, EM-kg, SKG3b, SNW (Keio University, Tokyo, Japan), and HeC1b (Kitazato University, Tokyo, Japan); the bladder cancer cell lines BC47, KU1, KU7, T24, and VMcub1 (Keio University); the renal cell cancer cell lines Kanai, RCC6, RCC7, RCC8, RCC9 (Surgery Branch, National Cancer Institute, Bethesda, MD), Caki, ACHN, 769P, 786O, and A498 (American Type Culture Collection); the prostate cancer cell lines LAC-4, PC3, DU145, and LNCaP; the breast cancer cell lines MCF7, MDA231 (Keio University); the lung cancer cell lines: LU399, large cell cancer, LK2, EBC1, and LC-1sq (squamous cell cancer), and SBC2, RER-LC-MA, RER-LC-OK, RER-LC-MS, VMRC-LCD, VMRC-LCP, ABC-1, LC-2, and A549/JCRB (adenocancer); the melanoma cell lines SKmel23, 886mel, A375mel, 928mel, 624mel, 586mel, 526mel, 624Amel, 397mel, and 1362mel (Surgery Branch, National Cancer Institute); the chronic myeloid leukemia cell line K562; the acute myeloid leukemia cell lines cell lines Kasumi-1 and HL60; the Burkitt's lymphoma cell line Daudi; and the T-cell lymphoma cell line, Mol14 (JCRB) were maintained in our laboratory. Cultured fibroblasts and activated T cells were generated from patients with informed consent at Keio University and cultured in RPMI with 10% fetal bovine serum. Melanocytes were kindly provided by Dr. Honjou (Morinaga Institute of Biological Science, Yokohama, Japan). Total RNA from normal tissues, including brain, heart, lung, stomach, small intestine, colon, pancreas, liver, kidney, bladder, prostate, testis, ovary, uterus, placenta, spleen, thymus, bone marrow, skeletal muscle, adipose tissue, retina, mammary gland, salivary gland, fetal brain, fetal liver, and fetal thymus, were purchased from Clontech (Palo Alto, CA). Total RNA from esophagus was purchased from Biochain Institute, Inc. (Hayward, CA). The pancreatic ductal adenocancer, malignant melanoma, endometrial cancer, stomach cancer, colon cancer, renal cell cancer, and lung cancer samples used in this study were surgically resected at Tokoku University hospital, Keio University Hospital, or the National Cancer Center, after informed consent was obtained according to the institution guidelines. These specimens were stored at −80°C until use.

**cDNA expression cloning with patient serum antibody (SEREX).** Normal testis tissue was obtained from a 76-year-old man who was clinically diagnosed with advanced prostate carcinoma (clinical stage D2) and had undergone bilateral castration at Keio University Hospital in 1998. Total RNA was extracted from human normal testis tissue using a guanidine isothiocyanate/CTC gradient. Polyadenylate + RNA was then purified twice with latex beads (PhastSep 30 super mRNA Purification kit; Takara Shuzo, Kyoto, Japan), and 5 μg of polyadenylate + RNA was used for construction of a cDNA library. First-strand synthesis was done using an oligo-dT primer with an internal XhoI cleavage site and 5'-methyl-CTP. The cDNA was ligated to EcoRI adapters and was digested with XhoI. These cDNA fragments were directly inserted into a zAP II (Stratagene, La Jolla, CA), bacteriophage expression vector, and packaged into phage particles, which were transformed in Escherichia coli. The primary size of the library was 1.2 × 10^6 plaques-forming units.

Immunoscreening with serum IgG antibody was done as previously described (16), using serum from a patient with pancreatic duct adenocarcinoma (stage IVb) who was treated at Tokoku University Hospital in 1998. Briefly, 1 mL of serum from the patient was diluted 1:15 with 5% skimmed milk and 0.1% sodium azide. This mixture was reacted with lysates of E. coli overnight and then diluted 1:200 in 5% skimmed milk in TBS. The testis cDNA library was expressed in XL-1 blue MRF strain with a titer of 1.5 × 10^10 per dish, and a total of 2.0 × 10^5 clones were screened. After DNA sequencing of the isolated clones, the clones were analyzed by comparison with genetic databases at the National Center for Biotechnology Information.

**Reverse transcription-PCR and Northern blot analysis.** Total RNAs were extracted from various cell lines and from tumor specimens by the guanidine isothiocyanate gradient method. Total RNAs derived from normal tissues were purchased from CLONTech Lab, Inc. (Palo Alto, CA). cDNAs were synthesized from 5 μg of total RNA by reverse transcriptase (Moloney murine leukemia virus Reverse Transcriptase RNase H−), Promega Corp., Madison, WI), and 30-cycle PCR was done at an appropriate annealing temperature for each primer set, with cDNA, an Ex Taq kit (Takara Shuzo), and the following primers: TW1, sense 5'-GTAAGGAGAAACACGATTCGAGGA-3', antisense 5'-CCAGCTTT-CACATTGTACGC-3'; TW2, sense 5'-AAGGATTCTTCTGCTGCTGTAACCC-3', antisense 5'-GGAAGACGGTAGAACGAGAAG-3'; TW3, sense 5'-CTTCGCGTCGCTCGTAC3'-antisense 5'-CTTCGCGTCGCTCGTAC3'-antisense 5'-CTTCGCGTCGCTCGTAC3'-antisense 5'-CTTCGCGTCGCTCGTAC3'-antisense 5'-CTTCGCGTCGCTCGTAC3'-antisense 5'-CTTCGCGTCGCTCGTAC3'-antisense 5'-CTTCGCGTCGCTCGTAC3'-antisense 5'-CTTCGCGTCGCTCGTAC3'-antisense 5'-CTTCGCGTCGCTCGTAC3'-antisense 5'-CTTCGCGTCGCTCGTAC3'-antisense 5'-CTTCGCGTCGCTCGTAC3'-antisense 5'-CTTCGCGTCGCTCGTAC3'.

Northern blot hybridization was done using total RNAs from cancer cell lines, normal tissues, and cultured normal cells: 10 μg per lane of total RNA was fractionated by electrophoresis in a 1.0% formaldehyde agarose gel and transferred to a nylon membrane (Hybond-XL, GE Healthcare, Little Chalfont, United Kingdom). The radioisotope was done using a BAS-2500 or BAS-5000 analyzer (FujiFilm, Tokyo, Japan).

**Detection of antigen-specific IgG antibodies in sera from patients with various cancers and from healthy individuals.** Screening of IgG antibodies specific for each antigen in sera from patients with various cancers and pancreatitis and from healthy individuals was done by an immunoscreening method similar to that used for the isolation of antigens. Informed consent was obtained from all the subjects. All sera were diluted 1:100, and each phage containing an antigen clone was mixed 2:1 with negative control phages that did not contain a DNA insert, to clarify the positive signals.
**Immunoprecipitation with a protein produced by in vitro transcription/translation.** In *vitro* transcription/translation was done using a Single Tube Protein System 3, T7 (Takara Shuzo). Briefly, isolated KU-CT-1L cDNA clone was amplified by 30 cycles of PCR using a Probed kit (Takara Shuzo) with sense primer 5′-GATTGCTAAGAAAGATATAAAGAA-GG-3′ and antisense primer 5′-TCATTGACCTGCAATGTTGTGTTA-3′. Incubation with End Conversion mix was done at 22°C for 5 minutes. Four units of T4 DNA ligase, piT2 Blue-2 Blunt Vector, and the reaction products were then mixed and incubated at 22°C for 1 hour. The cDNA template was amplified by 30 cycles of PCR using an Ex Taq kit (Takara Shuzo) with the 20-mer (5′-CAGCATGACCTGATTTGACG-3′) and the KU-CT-1L-specific antisense primer. Amplified cDNA template was used for *in vitro* transcription and translation reactions with 35S-labeled methionine. Translated KU-CT-1L proteins were separated using 10% SDS-PAGE, and the radioactivity was detected using a BAS-2500 or BAS-5000 analyser (Fujifilm).

Immunoprecipitation was done with *in vitro* translated 35S-labeled KU-CT-1L protein: 10 µl of sera were mixed with 2 µg of protein A-Sepharose CL-4B (Pharmacia, Inc., Piscataway, NJ) in TBS buffer [10 mmol/L Tris-HCl (pH 7.4), 140 mmol/L NaCl], incubated overnight at 4°C, and then washed in immunoprecipitation buffer [10 mmol/L Tris-HCl (pH 8), 500 µmol/L NaCl, 0.1% NP40] thrice. The pellet was resuspended in immunoprecipitation buffer and incubated with 5 µl of *in vitro* translated protein at 4°C for 2 hours. The resin was washed with immunoprecipitation buffer five times. The protein absorbed by the resin was dissolved in SDS gel-loading buffer and then incubated at 100°C for 5 minutes. The labeled KU-CT-1L protein was detected using a BAS-2500 or BAS-5000 analyzer (Fujifilm) after separation using 10% SDS-PAGE.

**Results**

**Isolation of a novel cancer testis antigen by SEREX using serum from a patient with pancreatic ductal adenocarcinoma.** To isolate cancer testis antigens, which are immunogenic in patients with pancreatic cancer, a total of 2.0 × 10⁶ clones from a normal testis cDNA library were immunoscreened by the SEREX method, using serum from a patient with pancreatic ductal adenocarcinoma. Three cDNA clones were isolated (Table 1). TW1 (KIAA1107) and TW2 [a zinc finger DAZ-interacting protein 1 (DZIP1), KIAA0996] were found to be expressed in various normal tissues, based on serial analysis of gene expression databases and reverse transcription-PCR analysis. However, reverse transcription-PCR analysis indicated that TW3 (located on chromosome 10p12 and giving hypo-heterologous protein FLJ32827, see below) was expressed only in testis tissue among 27 normal tissues and cultured noncancer cells, including fibroblasts, melanocytes, and activated T cells (a representative result is shown in Fig. 1A). However, TW3 was expressed in PK1 pancreatic cancer, MKN1 stomach cancer, TE4 esophageal cancer, Ishikawa endometrial cancer, RCC6 renal cell cancer, KIS lung adenocancer, and 526mel melanoma among the cancer cell lines tested and was expressed in pancreatic, endometrial, and lung cancers among the cancer tissues. Among the cell lines, TW3 was expressed in 2 of 8 pancreatic ductal adenocancer cell lines (Panc-1 and PK1); 2 of 5 stomach cancer cell lines (MKN1 and MKN46); 4 of 15 esophagus cancer cell lines (TE2, TE4, TE11, and TE12); 4 of 7 endometrial cancer cell lines (Ishikawa, EM-tn, Hec1b, and SNGW); 6 of 10 renal cancer cell lines (RCC6, RCC7, RCC8, Saito, ACHN, and A498); 1 of 4 prostate cancer cell lines (DU145); 7 of 12 lung cancer cell lines (adenocarcinoma: RERF-LC-MS, VMRC-LCD, VMRC-LCP, and A549; squamous cell carcinoma: EBC1, LK-2, and LC-1sq); 1 of 5 leukemia and lymphoma cell lines (K562); and 1 of 11 melanoma cell lines (526mel) but was not expressed in colon, bladder, and breast cancer cell lines. Among the cancer tissues tested, TW3 was expressed in 3 of 9 pancreatic ductal adenocarcinoma tissues (33%), 7 of 11 endometrial cancer tissues (64%), 1 of 8 renal cell cancer tissues (13%), and 9 of 24 lung cancer tissues (38%; Table 2). Northern blot analysis showed a main band of ~3.0 kb and a faint band of 1.5 kb in testis and cancer tissues (PA1 pancreatic cancer and EM2 endometrial cancer; Fig. 1B and C). These results indicate that TW3 is a novel cancer testis antigen that is frequently expressed in pancreatic cancers, endometrial cancers, and lung cancers. TW3 was subsequently renamed as KU-CT-1.

**Isolation and characterization of full-length KU-CT-1 cDNA.** The sequence of the isolated 1,452-base TW3 clone containing a stop codon and an additional polyadenylate signal in the 3′ region completely matched the sequence of the 1,223 bp at the 5′ end of a 2,831-bp hypothetical protein, FLJ32827. Comparison with genome databases showed that TW3 and FLJ32827 are splicing variants from a gene on chromosome 10p12 (Fig. 2). They seem to correspond to the 3.0-kb main band and 1.5-kb minor band detected by the Northern blot analysis, because KU-CT-1 encodes a protein of 397 amino acids and FLJ32827 contains 872 amino acids; these proteins were named KU-CT-1S (TW3) and KU-CT-1L (FLJ32827). The KU-CT-1 gene contains 20 exons over about 110 kb on chromosome 10p12, whereas KU-CT-1S includes of the first 11 exons of KU-CT-1, and KU-CT-1L has 19 exons (lacking exon 11 of KU-CT-1).

The first exon contained a sequence corresponding to the Kozak rule AGGATG sequence, and the last exon (exon 11 in KU-CT-1S and exon 20 in KU-CT-1L) contained a stop codon and an additional polyadenylate signal, AATAAA. KU-CT-1 does not have a leader sequence or a putative transmembrane domain, indicating that it is an intracellular protein. PROSITE analysis showed that the common region of KU-CT-1 has four Armadillo/β-catenin-like repeats, five N-glycosylation sites, a cyclic AMP–dependent and cyclic guanosine 3′,5′-monophosphate (GMP)–dependent protein kinase phosphorylation site, two protein kinase C phosphorylation sites, nine casein kinase

<table>
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<tr>
<th>Clone ID</th>
<th>Length (kb)</th>
<th>Identity</th>
<th>Accession number</th>
<th>Chromosomal location</th>
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<tr>
<td>TW2</td>
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<td>Zinc-finger protein DZIP1</td>
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<tr>
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<td>1.5</td>
<td>Hypothetical protein FLJ32827</td>
<td>NM173081</td>
<td>Chromosome 10</td>
</tr>
</tbody>
</table>

**Table 1. Antigens isolated by SEREX using a testis cDNA library and serum from a pancreatic cancer patient**
phosphorylation sites, and two tyrosine kinase phosphorylation sites. The additional COOH-terminal sequence of KU-CT1L contained two Armadillo/β-catenin-like repeats, three N-glycosylation sites, two cyclic AMP–dependent and cyclic GMP–dependent protein kinase phosphorylation sites, nine protein kinase C phosphorylation sites, 10 casein kinase phosphorylation sites, and two tyrosine kinase phosphorylation sites.

**Immunogenicity of KU-CT-1 in patients with various cancers.** The immunogenicity of KU-CT-1 was evaluated by screening for serum IgG antibody in patients with various cancers, using a phage plaque assay. The IgG antibody specific for KU-CT-1 was detected in sera from 3 of 20 pancreatic cancer patients, 2 of 12 endometrial cancer patients, 1 of 18 colon cancer patients, 1 of 10 prostate cancer patients, and 1 of 30 healthy individuals but was not detected in sera from nine esophageal cancer patients, 18 bladder cancer patients, 14 renal cell cancer patients, 22 melanoma patients, and seven pancreatitis patients (Table 3). To confirm the specificity of the antigen recognition, we further evaluated the IgG recognition of KU-CT-1 using an immunoprecipitation assay with a partial KU-CT-1 protein (448 amino acids of KU-CT-1S plus 36 amino acids in KU-CT-1L) produced by in vitro transcription/translation. Sera from patients who were antibody positive by the phage assay (three with pancreatic cancers, one with endometrial cancer, and one with prostate cancer) were also positive in the immunoprecipitation assay, whereas sera from two pancreatic-cancer patients and one healthy donor (HE17) who were antibody negative in the phage assay and one healthy donor who was antibody positive in the phage assay (HE27) were all negative in the immunoprecipitation assay (Fig. 3). Therefore, the results obtained in the phage plaque assay were consistent with those obtained in the immunoprecipitation assay, except in one healthy donor. The correlation between expression of KU-CT-1 and positive IgG was not evaluated, because of a lack of availability of paired samples, and this requires investigation in a future study. Nonetheless, the results indicate that KU-CT-1 was specifically recognized by sera from various cancer patients but not by sera from healthy individuals, indicating that KU-CT-1 is an immunogenic tumor antigen in cancer patients.

**Table 2. Frequency of KU-CT-1 mRNA expression in various cancer cell lines and tissues**

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Positive/total</th>
<th>Cell lines</th>
<th>Tissues</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreatic cancer</td>
<td>2/8 (25%)</td>
<td>3/9 (33%)</td>
<td></td>
</tr>
<tr>
<td>Colon cancer</td>
<td>0/7 (0%)</td>
<td>0/21 (0%)</td>
<td></td>
</tr>
<tr>
<td>Stomach cancer</td>
<td>2/5 (40%)</td>
<td>0/6 (0%)</td>
<td></td>
</tr>
<tr>
<td>Esophagus cancer</td>
<td>4/15 (27%)</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Endometrial cancer</td>
<td>4/7 (57%)</td>
<td>7/11 (64%)</td>
<td></td>
</tr>
<tr>
<td>Bladder cancer</td>
<td>0/5 (0%)</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Renal cell cancer</td>
<td>6/10 (60%)</td>
<td>1/8 (13%)</td>
<td></td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>1/4 (25%)</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Breast cancer</td>
<td>0/2 (0%)</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Lung cancer</td>
<td>7/12 (58%)</td>
<td>9/24 (38%)</td>
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<tr>
<td>Hematological malignancy</td>
<td>1/5 (20%)</td>
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<tr>
<td>Melanoma</td>
<td>1/11 (9%)</td>
<td>0/13 (0%)</td>
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**NOTE:** The results were obtained using 30 cycles of PCR. Abbreviation: ND, not determined.
Discussion

Early diagnosis and treatment of pancreatic cancer with conventional therapeutics is difficult, and new diagnostic and therapeutic methods are needed. Previous immunotherapy trials on patients with pancreatic cancer, including immunization with a mutated K-ras peptide along with granulocyte macrophage colony-stimulating factor administration, immunization with granulocyte macrophage colony-stimulating factor–transduced allogeneic pancreatic cancer cell lines along with adjuvant radiation, and chemotherapy following surgical excision have shown possible antitumor effects with detection of immune responses to tumor antigens (7, 17). However, detailed analysis of immune responses in pancreatic cancer for improvement of immunotherapy has not been done, partly because of the small number of tumor antigens available for the measurement of immune responses. Several pancreatic cancer antigens, including MUC-1, K-ras, HER-2/neu, and p53, have been reported, and the SEREX method has been used to identify hsp105, which is overexpressed in various cancers, including pancreatic cancer (18), and coactosin-like protein (CLP). CLP is ubiquitously expressed in normal tissues, but in induced HLA-A2-restricted, tumor-reactive CTLs, CLP(104-113) peptide–specific CTL activity was induced in three of five pancreatic cancer patients and in one of seven healthy donors, and a CLP-specific IgG antibody was detected in the sera of all seven cancer patients tested but not in nine healthy donors (19).

In this study, we isolated a novel cancer testis antigen KU-CT-1 by screening a testis cDNA library with serum from a patient with pancreatic ductal adenocarcinoma. Use of the testis cDNA library seems to be effective for isolation of cancer testis antigens by SEREX, because other cancer testis antigens (SSX2, SCP-1, and CAGE) have previously been isolated with sera from patients with melanoma, renal cell carcinoma, and gastric cancer, respectively. Using reverse transcription-PCR analysis, Kubuschok et al. reported the expression of 10 cancer testis antigens in pancreatic cancer: SCP-1, NY-ESO-1, SSX-1, SSX-2, SSX-4, GAGE, MAGE-3, MAGE-4, CT-7, and CT-8 (14). SSX-4 was expressed in 8 of 10 pancreatic cancer cell lines, and SCP-1 and GAGE were expressed in 29 and 13 of 61 pancreatic cancer tissues, respectively. Other studies evaluating the expression of cancer testis antigens indicate relatively rare expression of NY-ESO-1, Ctp11, MMA-1, XAGE1b, and HCA587 in pancreatic cancers (9–13). Because KU-CT-1 was expressed in 3 of 9 pancreatic cancer tissues (33%), it represents an additional cancer testis antigen that is expressed in pancreatic cancers. Furthermore, KU-CT-1 was shown to be immunogenic in multiple patients with pancreatic cancers, indicating possible use of KU-CT-1 for diagnosis and immunotherapy in patients with pancreatic cancers. In addition, KU-CT-1 was found to express even more frequently in endometrial cancers (7 of 11 patients, 64%) and lung cancers (9 of 24 patients 38%) and was immunogenic in some of these patients, indicating that it may also be useful for immunotherapy in patients with endometrial cancer and lung cancers.

The KU-CT-1 gene consists of 20 exons and is located on chromosome 10p12. It is transcribed to give two splice variants

![A](image1.png)  ![B](image2.png)

**Fig. 2.** Nucleotide and amino acid sequences of KU-CT-1S and KU-CT-1L. A, nucleotide and amino acid sequences of KU-CT-1S. B, nucleotide and amino acid sequences of KU-CT-1L. KU-CT-1S and KU-CT-1L share the same sequence from 1 to 1,214 at the NH2-terminus. Position 1,214 (A), KU-CT-1 contains 20 exons and is localized in chromosome 10p12. KU-CT-1S contains 11 of these exons (exons 1-11), and KU-CT-1L contains 19 exons (lacking exon 11). Armadillo/h-catenin-like repeat sequences are underlined.

**Table 3.** The presence of an IgG antibody specific for KU-CT-1 in sera from various cancer patients

<table>
<thead>
<tr>
<th>Subject</th>
<th>Positive/total</th>
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<tbody>
<tr>
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<tr>
<td>Colon cancer</td>
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<td>Endometrial cancer</td>
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<td>Melanoma</td>
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<tr>
<td>Pancreatitis</td>
<td>0/7</td>
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<tr>
<td>Healthy individual</td>
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</tbody>
</table>
of 1.5 and 2.8 kb, which encode for proteins of 397 amino acids (KU-CT-1S) and 872 amino acids (KU-CT-1L), respectively. Northern blot analysis indicated that KU-CT-1L is the major product, and the expression pattern of the splice variants was similar between normal testis tissue and various cancers. The function of KU-CT-1 remains unknown with regard to oncogenesis and cancer progression. KU-CT-1L does not possess a transmembrane domain and has four Armadillo/β-catenin-like repeats that are typically present in cytoplasmic proteins, including β-catenin, adenomatosus polyposis coli (APC) gene, and a melanoma antigen KU-MEL-1 (20). It is assumed that three of the Armadillo/β-catenin-like repeats make one positively charged helix that allows interaction with other proteins. The functions of two cancer testis antigens are reported. SCP-1 is involved in the pairing of homologous chromosomes, an essential step for the generation of haploid cells in meiosis I (21), and MAGE-A1 binds to SKIP, inhibiting the activity of a SKIP-interacting transactivator, and recruits HDACs, thereby acting as a potent transcriptional repressor (22). Thus, the regulation of transcription by MAGE-A1 may be involved in the oncogenesis and tumor progression of cancer cells. Several cancer testis antigens, including SSX, NY-ESO-1, and N-RAGE, were reported to be expressed in mesenchymal stem cells and down-regulated after differentiation (23), suggesting possible expression of cancer testis antigens in cancer stem cells. Therefore, further investigations are necessary for the functional role of KU-CT-1 in the formation of malignant phenotype of cancer cells and its expression in cancer stem cells as well.

Serum IgG specific for KU-CT-1 was detected in patients with pancreatic cancer and endometrial cancer, in which KU-CT-1 is frequently expressed. Correlation between the expression of KU-CT-1 and the presence of IgG was not studied because paired samples were not available. However, the presence of an IgG antibody against KU-CT-1 in multiple patients suggests that KU-CT-1 activated CD4+ helper T cells (Th) in patients with these cancers, suggesting that KU-CT-1 may at least be useful as a CD4+ T-cell antigen for immunotherapy in patients with these cancers and particularly in patients with antibody-positive serum. In addition, many SEREX-derived antigens have been shown to induce CD8+ CTLs (24–28), and a positive correlation was observed between positive serum IgG antibody and induction of CD8+ CTLs against a cancer testis antigen, NY-ESO-1 (24, 29). Therefore, KU-CT-1 may be a useful target for immunotherapy in patients with pancreatic cancers and endometrial cancers.

In summary, screening of a testis cDNA library with serum from a patient with pancreatic cancer led to identification of a novel cancer testis antigen, KU-CT-1, which was frequently expressed in pancreatic cancer, lung cancer, and endometrial cancer and was immunogenic in some of these patients. These results indicate that KU-CT-1 may be useful diagnostically and in immunotherapy for patient with these cancers.

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References

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