Jpn. J. Cancer Res. **91**, 239–247, February 2000

Mouse Homologue of the Human *SART3* Gene Encoding Tumor-rejection Antigen

Kenji Harada,[1] Akira Yamada,[1, 2, 3] Takashi Mine,[2] Nobutoshi Kawagoe,[1] Hideo Takasu[1] and Kyogo Itoh[1, 2]

[1]Department of Immunology, Kurume University School of Medicine and [2]Cancer Vaccine Development Division, Kurume University Research Center for Innovative Cancer Therapy, Asahi-machi 67, Kurume, Fukuoka 830-0011

We recently isolated a human *SART3* (*hSART3*) gene encoding a tumor-rejection antigen recognized by HLA-A2402-restricted cytotoxic T lymphocytes (CTLs). The hSART3 was also found to exist as an RNA-binding nuclear protein of unknown biological function. In this study, we cloned and analyzed the homologous mouse *SART3* (*mSART3*) gene in order to understand better the function of hSART3, and to aid in establishing animal models of specific immunotherapy. The cloned 3586-bp cDNA encoded a 962-amino acid polypeptide with high homology to *hSART3* (80% or 86% identity at the nucleotide or protein level, respectively). Nonapeptides recognized by the HLA-A2402-restricted CTLs and all of the RNA-binding motifs were conserved between hSART3 and mSART3. The *mSART3* mRNA was ubiquitously expressed in normal tissues, with low level expression in the liver, heart, and skeletal muscle. It was widely expressed in various organs from as early as day 7 of gestation. *mSART3* was mapped to chromosome 5, a syntenic region for human chromosome 12q23-24, and its genomic DNA extended over 28-kb and consisted of 19 exons. This information should be important for studies of the biological functions of the SART3 protein and for the establishment of animal models of specific cancer immunotherapy.

Key words: SART3 — Tumor-rejection antigen — RNA-binding protein — HLA-A2402 — Cytotoxic T lymphocytes

One of the most significant advances in the field of tumor immunology has been the recent identification of genes encoding tumor-rejection antigens that are recognized by HLA-class-I-restricted and tumor-specific cytotoxic T lymphocytes (CTLs).[1, 2] The potential application of these findings to the development of cancer vaccines has brought nearer the prospect of specific cancer immunotherapy. Indeed, several peptides encoded by these genes are now under clinical trial as cancer vaccines, and major tumor regression has been observed in some melanoma patients.[3, 4] We have recently isolated a human *SART3* (*hSART3*) gene encoding a tumor-rejection antigen from cDNA of a human esophageal cancer cell line.[5] Two SART3-derived peptides, at positions 109–118 and 315–323, that are recognized by HLA-A2402-restricted CTLs, are able to induce HLA-A2402-restricted and tumor-specific CTLs from peripheral blood mononuclear cells (PBMCs) of HLA-A24 positive (HLA-A24$^+$) cancer patients. *hSART3* encodes a *Mr* 140 000 protein expressed in both the cytosol of the majority of proliferating cells, including non-tumorous cell lines, and the nucleus of the majority of cancer cells. However, the SART3 protein was undetectable in normal tissues except for the testis and fetal liver, regardless of its ubiquitous expression at the mRNA level.[5] There are several motifs in the sequence of the SART3 protein: nuclear localization signals[6] around positions 612–615 and 641–647, a ribonucleoprotein-1 (RNP-1) motif,[7] one of the well-characterized RNA-binding motifs, at positions 746–753 and 841–848, and a tyrosine phosphorylation motif[8] at positions 309–316. The involvement of the SART3 protein in tyrosine phosphorylation (data not shown) and the RNA-binding capacity of the SART3 protein[9] have both been demonstrated. These results suggest that the *hSART3* gene plays an important role in cellular proliferation. However, its biological function remains unknown. To understand better the biological function of hSART3 and to aid in establishing animal models of cancer immunotherapy, we have cloned a mouse homologue (*mSART3*) of the *hSART3* gene, and here report the characterization, expression pattern, chromosomal localization, and genomic organization of this homologue.

MATERIALS AND METHODS

Homology searches and cloning of the *mSART3* gene The *hSART3* cDNA sequence was used as a query in BLAST2 homology searches against mouse Expressed sequence tag (EST) databases. An EST clone AA674246 was obtained from Genome Systems (St. Louis, MO). A mouse brain cDNA library (TaKaRa, Otsu) was screened

[3] To whom correspondence should be addressed at the Department of Immunology, Kurume University School of Medicine, Asahi-machi 67, Kurume, Fukuoka 830-0011.
E-mail: akiymd@med.kurume-u.ac.jp

Sequence data from this article have been deposited with the GenBank Data Library under Accession No. AF172722.

with a 0.8-kbp cDNA fragment of the EST clone AA674246. Positive colonies were selected with an additional round of screening. Additionally, the 5′ rapid amplification of cDNA ends (RACE) polymerase chain reaction (PCR) method with a mouse 13.5-day embryo cDNA library was used to obtain a full-length cDNA. Both strands of cloned cDNAs were sequenced using an ABI 377 automated sequencer (Applied Biosystems, Foster City, CA) and BigDye Terminator chemistry (Applied Biosystems) by the primer-walking method. All oligonucleotides were purchased from Hokkaido System Science (Sapporo). Alignment against the sequences of some homologues was performed using the Matchbox program (http://www.fundp.ac.be/sciences/biologie/bms/matchbox_submit.html).

Northern blot analysis Total RNAs were isolated using the acid guanidium thiocyanate-phenol-chloroform method.[10] The RNA samples (5 or 10 μg/lane) were electrophoresed on 1% agarose gels in the presence of formaldehyde, transferred to a nylon membrane (Hybond-N^+; Amersham Pharmacia Biotech, Uppsala, Sweden), and then fixed with UV crosslinker (Spectronics, Westbury, NY). The mouse embryo multiple tissues northern blot filter was purchased from Clontech (Palo Alto, CA). The fragment of AA674246 was radiolabeled with a Multiprime DNA labelling system (Amersham Pharmacia Biotech) and used as a probe. A 1.1-kbp fragment of *G3PDH* (glyceraldehyde-3-phosphate dehydrogenase) cDNA (Clontech) was also used as a control. The membrane was hybridized overnight at 65°C in a hybridization buffer (7% sodium dodecylsulfate (SDS), 1 m*M* EDTA, 0.5 *M* Na_2HPO_4, pH 7.2) with ^{32}P-labeled probes. The membranes were washed twice at 65°C with a washing buffer (1% SDS, 40 m*M* Na_2HPO_4, pH 7.2), and then autoradiographed. The relative expression level of *mSART3* mRNA was calculated by use of the following formula: index = (*G3PDH* density of a standard sample in each filter/*G3PDH* density of a sample) × (*SART3* density of a sample /*SART3* density of the standard sample in each filter.

Chromosomal mapping Chromosomal localization of *mSART3* was determined by PCR analysis of a mouse/hamster radiation hybrid panel (Research Genetics, SW Huntsville, AL). This panel of 100 radiation hybrid clones of the whole mouse genome was created by fusing irradiated mouse embryo primary cells (129aa) with hamster recipient cells (A23). The 3′-untranslated region primers (5′-AATGGGAGAGCTTGTCACTC-3′ and 5′-ACAAAACTCAATGGGGAGAC-3′), which generate a 320-bp DNA fragment, were used under the following conditions: 94°C for 2 min, 40 cycles at (94°C for 30 s, 56°C for 30 s and 72°C for 30 s), and 72°C for 7 min. The occurrences of PCR products in each clone were analyzed by 2% agarose gel elecrophoresis with ethidium bromide staining. The mapping results were analyzed on the radiation hybrid mapping server of the mouse genome at the Whitehead Institute/MIT Center (http://www.genome.wi.mit.edu/cgi-bin/mouse_rh/rhmap-auto/rhmapper.cgi).

Isolation of genomic DNA The *mSART3* genomic DNA fragments were obtained by PCR from BALB/c mouse genomic DNA. Oligonucleotide primer pairs used for the PCR were designed from the *mSART3* cDNA sequence. One primer pair was 5′-GCGCAAGATGGCGACGACGG-3′ and 5′-CGTACTTGGCATTCCCTCTG-3′, and the other was 5′-GAGCTCTGGGACAGCATCAT-3′ and 5′-ACAAAACTCAATGGGGAGAC-3′. The isolated fragments were subcloned into a vector pCR-XL-TOPO (Invitrogen, Carlsbad, CA), and obtained clones were subsequently sequenced with gene-specific primers. The exon-intron junctions were determined by comparing the sequence with the *mSART3* cDNA sequence using GeneWorks software (Intelligenetics, Mountain View, CA). Introns were further amplified with appropriate primers and their sizes were estimated.

RESULTS

Isolation of *mSART3* cDNA Several mouse cDNA clones containing nucleotide sequences with high similarity to the *hSART3* cDNA were found in the EST database. The EST clone AA674246 showed the highest homology, and was therefore used as a probe to clone the mouse homologue of the *hSART3* gene. A 3586-bp cDNA clone was obtained from a mouse brain cDNA library by means of a combination of cDNA library screening with probe hybridization and 5′-RACE. The sequence flanking the presumed start site ($^{+12}$AAGATGGCG^{+20}) has the features of the Kozak consensus sequence for a eukaryotic translation start site.[11, 12] There is a typical ATTAAA polyadenylation signal at positions 3566–3571 in the 3′ untranslated region (UTR).[13] As shown in Fig. 1A, the cloned *mSART3* showed 80% identity to *hSART3* at the nucleotide level, and had one major open reading frame (ORF) covering a region from nucleotide 15 to 2900, encoding a 962-amino acid (aa) polypeptide that had 86% homology to hSART3.[5] Amino acid sequences capable of inducing HLA-A24 restricted CTLs at positions 316–324, RNP-1 motifs at positions 746–753 and 841–848, nuclear localization signals around positions 613–616 and 642–648, and the tyrosine phosphorylation motif at positions 308–315 were all conserved in both hSART3 and mSART3 (Fig. 1A).

A BLAST2 search and alignment showed that mSART3 had 25, 26, and 16% similarity to a *Caenorhabditis elegans* ORF of 836 aa (Accession No. CAA97405), an *Arabidopsis thaliana* ORF of 768 aa (CAB45062), and a *Drosophila melanogaster* ORF of 943 aa (CAA75535),[14, 15] respectively, at the amino acid level. Highly conserved regions at the C-terminus are shown in Fig. 1B. These ORFs also contained RNP-1 motifs.

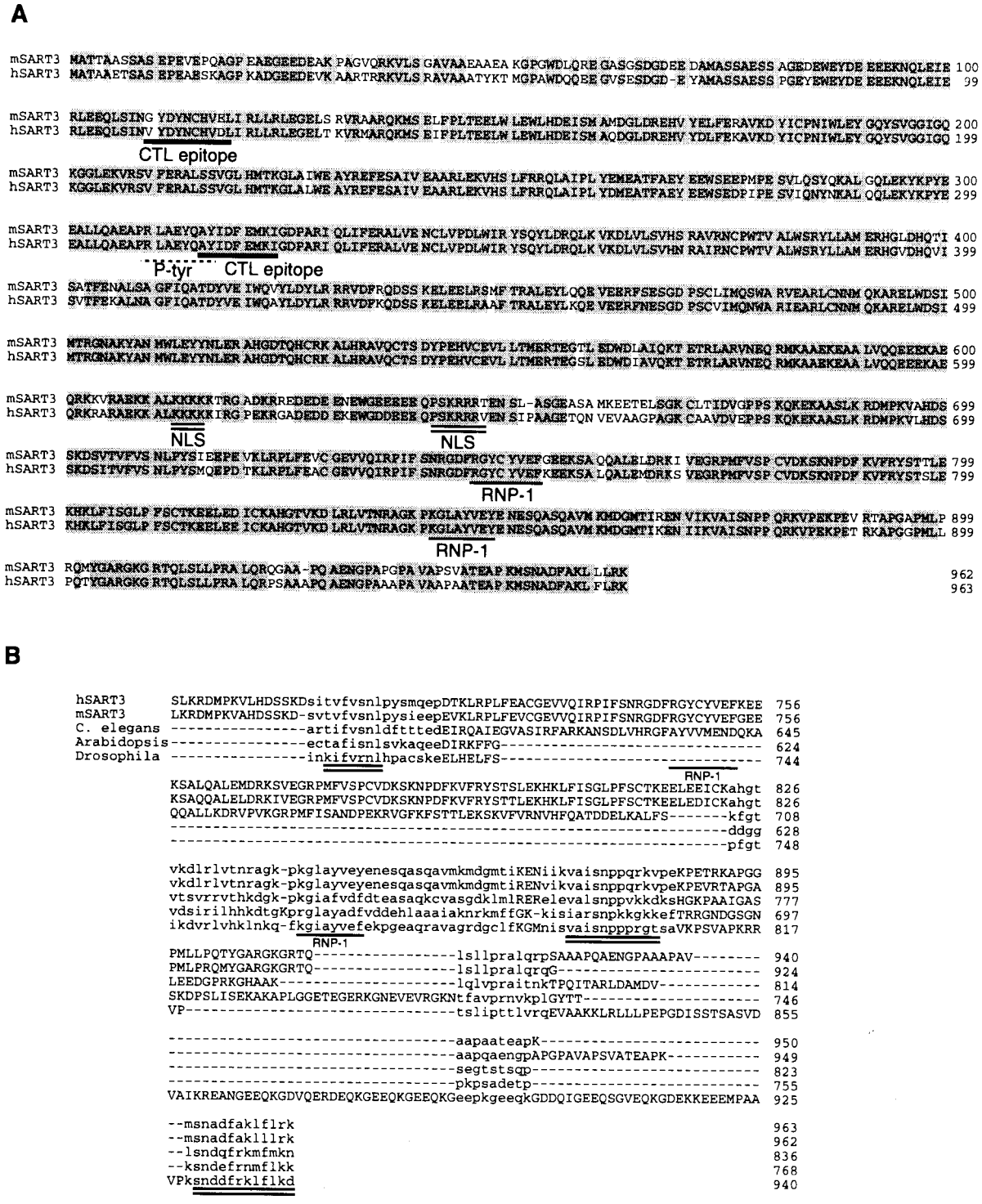

Fig. 1. Deduced amino acid sequence of the *mSART3* gene and comparison with those of homologues. (A) Alignment with the hSART3 protein. Identical amino acids are indicated by shading. Two hSART3-derived peptides (CTL epitopes) recognized by HLA-A2402-restricted CTLs are underlined. The following putative motif sites are also underlined: nuclear localization signal (NLS), RNP-1, and tyrosine phosphorylation motif (P-tyr). (B) Multiple alignment with the C-terminal regions of putative SART3 proteins of humans, mice, *C. elegans*, *A. thaliana*, and *D. melanogaster*. Highly conserved regions are double-underlined. RNP-1 motif sites are also indicated. Amino acid positions are shown on the right.

***mSART3* mRNA expression** Expression of the *mSART3* mRNA in various tissues of adult mice was analyzed by northern blot analysis. *mSART3* mRNA was ubiquitously expressed in adult mouse tissues, and a single species of message (approximate size, 3.6 kb) was observed (Fig. 2A). Relative expression levels of the mRNA ranged between 0.1 and 1.1, when the expression level in the thymus was considered to be 1.0. mRNA expression was high in the testis (1.0), thymus (1.0), spleen (1.1), and lung (1.0), but low in the liver (0.2), heart (0.2), and skeletal muscle (0.1). Expression of the *mSART3* mRNA was further analyzed at various developmental stages. It was expressed from the early prenatal stages — as early as day 7 of gestation (E7) — and increased thereafter (Fig. 2B). The expression of *mSART3* mRNA was detected in all the organs tested at different embryonic stages (gestational days 14, 16, 18; E14, E16, E18) and at postnatal day 1 (P1). The level of expression of *mSART3* was relatively

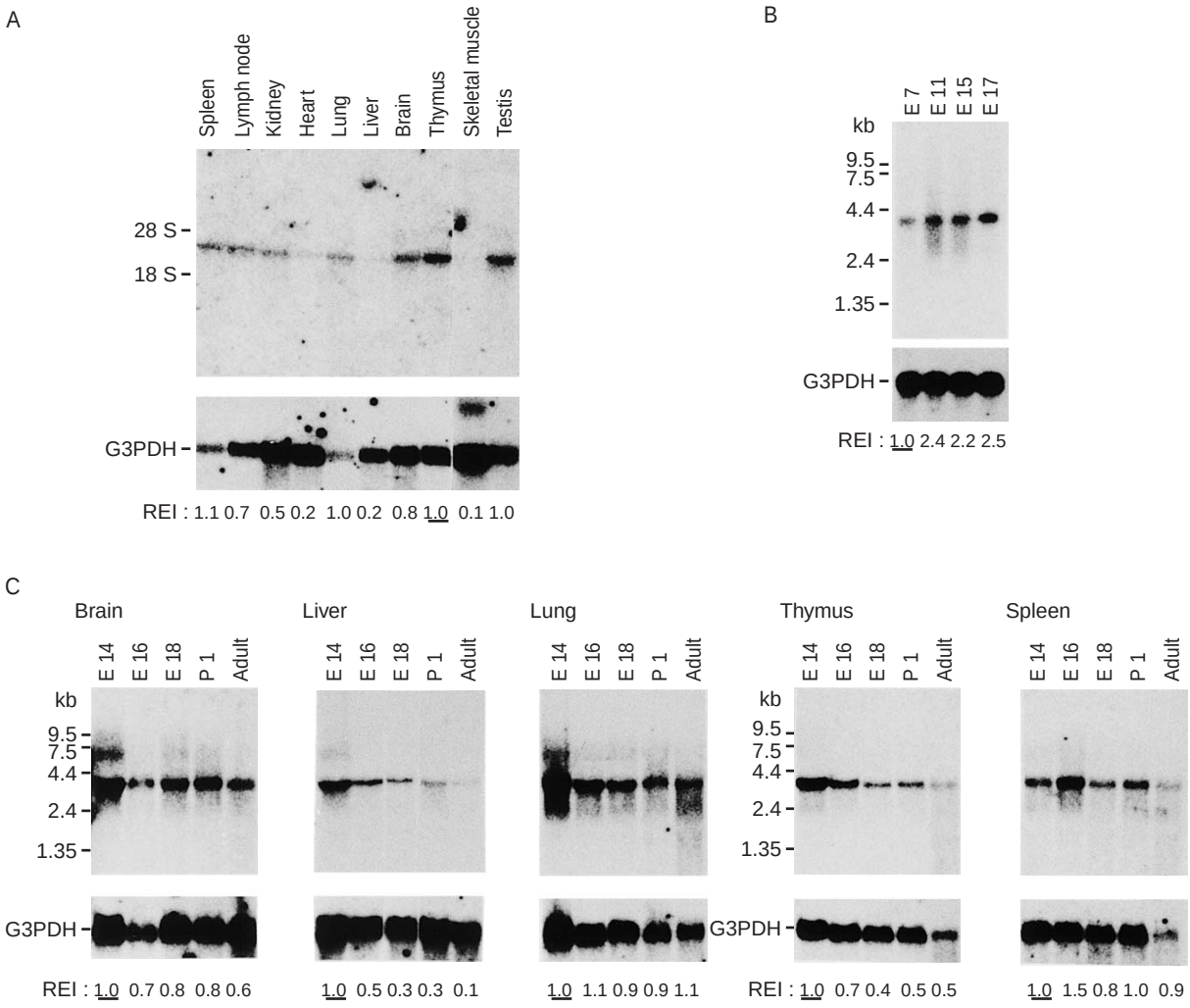

Fig. 2. Northern blot analyses of *mSART3* gene expression. (A) Tissue distribution of the *mSART3* transcript. A northern blot with 10 μg of total RNA from each indicated mouse tissue was probed with a *mSART3* cDNA fragment. The same blot was hybridized with a *G3PDH* probe to normalize the amount of RNA loaded in each lane. The relative expression level of *mSART3* mRNA was calculated by means of the following formula: Relative expression index (REI) = (*G3PDH* density of a standard sample in each filter / *G3PDH* density of a sample) × (*SART3* density of a sample / *SART3* density of the standard sample. The standard sample is underlined. (B) *mSART3* expression in different embryonic developmental stages. A blot with 2 μg of poly(A)$^+$ RNA from each indicated period of embryonic development (E) was probed and the REI was calculated. (C) *mSART3* expression in major or lymphoid organs at different embryonic stages. A northern blot with 5 μg or 10 μg of total RNA from each embryonic (E) and postnatal (P) stage of organs was probed and the REI was calculated. The positions of RNA molecular weight standards are indicated on the left.

constant during developmental stages in the brain, lung and spleen, whereas it slightly declined in the thymus, and was greatly reduced in the liver (Fig. 2C).

Chromosomal localization of *mSART3* Chromosomal location of the *mSART3* gene was determined by radiation hybrid mapping. The *mSART3* was located on chromosome 5, 14.3 cR distal from the D5Mit317 marker gene (Fig. 3). This position is syntenic to the human chromosome 12q23-24, to which the *hSART3* has been mapped (Accession No. D63879/SGC31638).

Genomic Structure of *mSART3* Genomic DNA of *mSART3* gene was amplified by PCR, and a total of approximately 28 kb of genomic DNA was partially sequenced to determine the intron-exon structure of the *mSART3* gene. As shown in Fig. 3, the *mSART3* mRNA was a multiply spliced transcript consisting of at least 19 exons and 18 introns within the sequenced region. The exon-intron structure and boundary sequences of *mSART3* are presented in Table I.

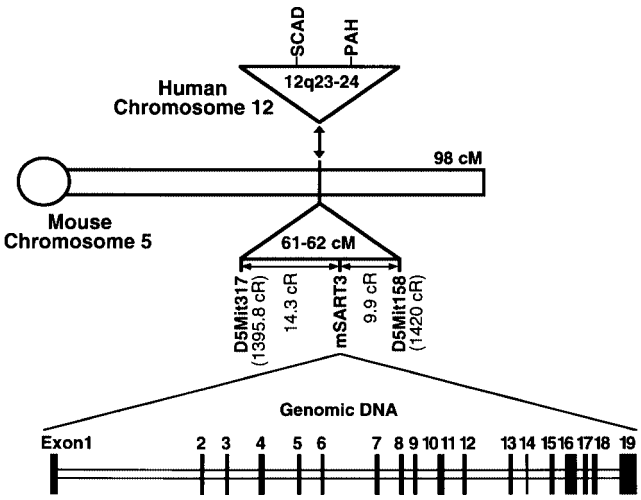

Fig. 3. Chromosomal localization and genomic structure of the *mSART3* gene. Relevant markers on the mouse chromosome 5 are shown. The distances between the loci are listed in centiRays (cR). The location of *hSART3* syntenic to *mSART3* is indicated in the upper part. The *SCAD* (short-chain acyl-CoA dehydrogenase) and *PAH* (phenylalanine hydroxylase) genes, which cause metabolic inherited diseases, are located in the indicated regions. The genomic structure of *mSART3* is shown in the lower part. The relative positions of the *mSART3* exons on the genome are indicated with appropriate scales.

DISCUSSION

The *mSART3* gene was highly homologous to *hSART3* at both the nucleotide and protein levels. Peptide sequences capable of inducing HLA-A24-restricted CTLs, RNP-1 motifs, nuclear localization signals, and a tyrosine

Table I. Genomic Structure and Boundary Sequences of *mSART3*

Exon	Size	Position in cDNA	Intron size	Splice acceptor[a)]	Splice donor[a)]
1	322	8– 329	~6.9 kb[b)]	—	CAG**gt**tggccgg
2	127	330– 456	~1.1 kb[b)]	tgctctt**ag**CTG	AAG**gt**aagctgc
3	105	457– 561	~1.5 kb[b)]	tgtctct**ag**AGC	TCT**gt**gagtgcc
4	185	562– 746	~1.7 kb[b)]	gtcttttagGTC	CGG**gt**gagtcca
5	52	747– 798	957 bp	gattcac**ag**CTG	ACG**gt**aagagca
6	125	799– 923	~2.5 kb[b)]	tttctgt**ag**AGA	CTG**gt**gagtgcc
7	156	924–1079	998 bp	gtgctgc**ag**CTG	CTA**gt**aagataa
8	139	1080–1218	527 bp	ttgtcat**ag**GAT	CTG**gt**gtgaacg
9	108	1219–1326	~1.1 kb[b)]	gcattgc**ag**CGA	AGG**gt**acagttg
10	78	1327–1404	118 bp	ttccaac**ag**ACT	AGC**gt**aagtggc
11	59	1405–1463	901 bp	ttttgga**ag**GTT	GAG**gt**gagcgtt
12	110	1464–1573	~2.2 kb[b)]	gtgctt**ag**GCT	ACG**gt**gagtgaa
13	113	1574–1686	~0.7 kb[b)]	ctgctct**ag**GGC	AAG**gt**gggagcc
14	77	1687–1763	~1.1 kb[b)]	tctctcc**ag**GGA	AAG**gt**gacactg
15	169	1764–1932	592 bp	cattcct**ag**GCC	AAG**gt**accgttg
16	452	1933–2384	380 bp	tgcctgc**ag**AGC	AAG**gt**atgcttt
17	153	2385–2537	313 bp	tgtttgc**ag**GTG	AAG**gt**gagtggg
18	191	2538–2728	~1.1 kb[b)]	gtgtgtt**ag**GGC	CGC**gt**aaagtgt
19	835	2729–3563	—	ctcttct**ag**GCG	—

a) Exon sequences are shown in uppercase letters, intron sequences are shown in lowercase letters, and invariant ag (splice acceptor) and gt (splice donor) nucleotides are shown in boldface type.

b) Approximate size based on agarose gel electrophoretic mobility of intron-spanning PCR products relative to standards.

phosphorylation motif were all conserved in both the hSART3 and mSART3 (Fig. 1A). In addition to mSART3, putative SART3 homologues were found in *C. elegans*, *A. thaliana*, and *D. melanogaster*. These genes also have an RNP-1 motif in the COOH-terminal (Fig. 1B). Physiological roles of RNP motif proteins have been discerned from the consequences of loss of expression or mutations in the RNP proteins that result in developmental disorders in humans and other organisms.[16–22] A deletion mutant of 4f-rnp, a putative *SART3* homologue of *Drosophila*, has been reported to have a lethal effect.[14] These facts suggest that the SART3 protein plays critical roles in the development and maintenance of various organs, although its biological functions remain to be clarified.

RNP motif proteins, such as La proteins, are common targets in autoimmune disease, especially in patients with systemic lupus erythematosus.[23–28] Many autoantigens seem to be components of subcellular nuclear particles involved in important cell functions, such as DNA replication,[29, 30] DNA transcription,[31–33] RNA processing,[34] and cell division.[35–37] Autoantibodies are often detectable in cancer patients, although their molecular bases have not been well clarified.[38–43] We previously identified the *SART1* gene as encoding a tumor-rejection antigen recognized by CTLs.[44] The same gene was also reported as encoding a self antigen that elicits IgE antibody reponse.[45] The SART1 protein is localized in the nucleus and has DNA-binding capacity (data not shown). We reported hSART3 as a tumor rejection antigen,[5] whlie Gu *et al*.[9] reported it as an RNA-binding nuclear protein. These results suggest that the SART3 antigen is a possible self antigen eliciting autoantibodies. The molecular mechanisms involved in this phenomenon should be elucidated.

mSART3 was mapped to mouse chromosome 5, 14.3 cR distal from the D5Mit317 marker gene, which is a region syntenic to the human chromosome, 12q23-24, that includes the *hSART3* gene. Some of the mouse genes around this region have already been mapped, and each human counterpart was localized to 12q23-24.[46–48] The human chromosome 12q23-24 is one of the best-characterized regions of the human genome. Several genes mapped on this region are involved in inherited diseases, including many kinds of metabolic diseases (Fig. 3).[46, 49–51] This 12q23-24 region is also involved in the other diseases for which the candidate gene has not yet been identified: Brachydactyly type C,[52] Noonan syndrome-I,[53] and spinal muscular atrophy.[54–56] In addition, this region is known to be rearranged in a variety of cancers (e.g., chronic lymphoproliferative disorders, non-Hodgkin's lymphomas, germ cell tumors, and astrocytomas).[57] No phenotype or disease loci associated with this region has yet been reported on the mouse chromosome.

In this study, we isolated the *mSART3* genomic DNA and determined the intron-exon boundaries of the *mSART3* gene. Two *hSART3* genomic DNA clones (Accession Nos. AQ395743 and B83376), which contained sequences around intron-exon junctions, were obtained from the database. These boundaries in the *hSART3* gene were found at positions equivalent to those observed in the *mSART3* gene. All *mSART3* introns belonged to the predominant vertebrate splicing GT-AG class of introns. Both the *mSART3* cDNA and its genomic clone should be novel models for improving our understanding of the molecular basis of these diseases.

The expression of *mSART3* at the mRNA level was ubiquitous in normal tissues with high-level expression in the spleen, thymus, lymph node, lung, testis, and brain, and low levels in the liver, heart, and skeletal muscle. The *hSART3* mRNA was also ubiquitously expressed in normal tissues, whereas the expression was low in the thymus and PBMCs.[5] The meaning of the difference of expression in the lymphoid organs between mouse and human *SART3* gene is unclear. The mRNA expression of both the *mSART3* and *hSART3* in the testis was high. A similar expression pattern, i.e., ubiquitous with high-level expression in the testis, was previously reported for *SART1*.[44] Although these genes are ubiquitously expressed in normal and malignant cells or tissues at the mRNA levels, protein expression levels in the normal and malignant cells were very different. Expression of the SART3 protein as well as SART1 43-kD protein was limited in the malignant tumor cells or well proliferating cells, and undetectable in the normal adult tissues except for the testis.[5]

Two peptides, $hSART3_{109-118}$ and $hSART3_{315-323}$, encoded by the *hSART3* gene were recognized by HLA-A2402-restricted CTLs and were able to induce HLA-A2402-restricted and tumor-specific CTLs in PBMCs of cancer patients.[5] Thus, SART3 and its peptides at positions 109–118 ($hSART3_{109-118}$) and 315–323 ($hSART3_{315-323}$) are appropriate molecules for use in specific immunotherapy for HLA-$A24^+$ patients with various histological types of cancer. Because of the expression of SART3 proteins in the normal testis, the testis is a possible target organ of collateral effects of the specific immunotherapy. It should be noted that no severe collateral effect in the testis has been reported in the clinical trials of melanoma antigen (MAGE) specific immunotherapy against melanoma patients, although the *MAGE* gene family proteins are similarly expressed in the normal testis.[4, 58]

The anchor motifs on the antigenic peptides to bind mouse class I major histocompatibility complex, H-$2K^d$, are similar to those for the human HLA-A2402,[59] and the sequence of the $hSART3_{315-323}$ peptide is conserved in mSART3. Moreover, fourteen different peptides with H-$2K^d$ binding motifs were also found in mSART3. These results suggest that mSART3 and its peptides should be useful as novel tools for developing animal models of specific cancer immunotherapy.

ACKNOWLEDGMENTS

This study was supported in part by Grants-in-Aid 08266266, 09470271, 10153265, 09770985, 10671230, and 09671401 from the Ministry of Education, Science, Sport and Culture (Japan) and Grant H10-genome-003 from the Ministry of Health and Welfare (Japan).

(Received September 30, 1999/Revised November 4, 1999/ Accepted November 10, 1999)

REFERENCES

1) Boon, T. and van der Bruggen, P. Human tumor antigens recognized by T lymphocytes. *J. Exp. Med.*, **183**, 725–729 (1996).
2) van der Bruggen, P., Traversari, C., Chomez, P., Lurquin, C., De Plaen, E., Van den Eynde, B., Knuth, A. and Boon, T. A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. *Science*, **254**, 1643–1647 (1991).
3) Rosenberg, S. A., Yang, J. C., Schwartzentruber, D. J., Hwu, P., Marincola, F. M., Topalian, S. L., Restifo, N. P., Dudley, M. E., Schwarz, S. L., Spiess, P. J., Wunderlich, J. R., Parkhurst, M. R., Kawakami, Y., Seipp, C. A., Einhorn, J. H. and White, D. E. Immunologic and therapeutic evaluation of a synthetic peptide vaccine for the treatment of patient with metastatic melanoma. *Nat. Med.*, **4**, 321–327 (1998).
4) Nestle, F. O., Alijagic, S., Gilliet, M., Sun, Y., Grabbe, S., Dummer, R., Burg, G. and Schadendorf, D. Vaccination of melanoma patients with peptide- or tumor lysate-pulsed dendritic cells. *Nat. Med.*, **4**, 328–332 (1998).
5) Yang, D., Nakao, M., Shichijo, S., Sasatomi, T., Takasu, H., Matsumoto, H., Mori, K., Hayashi, A., Yamana, H., Shirouzu, K. and Itoh, K. Identification of a gene coding for a protein possessing shared tumor epitopes capable of inducing HLA-A24-restricted cytotoxic T lymphocytes in cancer patients. *Cancer Res.*, **59**, 4056–4063 (1999).
6) Hicks, G. R. and Raikhel, N. V. Protein import into the nucleus: an integrated view. *Annu. Rev. Cell Dev. Biol.*, **11**, 155–188 (1995).
7) Christopher, G. B. and Gideon, D. Conserved structures and diversity of functions of RNA-binding proteins. *Science*, **265**, 615–621 (1994).
8) Patschinsky, T., Hunter, T., Esch, F. S., Cooper, J. A. and Sefton, B. M. Analysis of the sequence of amino acids surrounding sites of tyrosine phosphorylation. *Proc. Natl. Acad. Sci. USA*, **79**, 973–977 (1982).
9) Gu, J., Shimba, S., Nomura, N. and Reddy, R. Isolation and characterization of a new 110 kDa human nuclear RNA-binding protein ($p110^{nrb}$). *Biochim. Biophys. Acta*, **1399**, 1–9 (1998).
10) Chomczynski, P. and Sacchi, N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.*, **162**, 156–159 (1987).
11) Kozak, M. Bifunctional messenger RNAs in eukaryotes. *Cell*, **47**, 481–483 (1986).
12) Kozak, M. Recognition of AUG and alternative initiator codons is augmented by G in position +4 but is not generally affected by the nucleotides in position +5 and +6. *EMBO J.*, **16**, 2482–2492 (1997).
13) Swimmer, C. and Shenk, T. Selection of sequence elements that substitute for the standard AATAAA motif which signals 3′ processing and polyadenylation of late simian virus 40 mRNAs. *Nucleic Acids Res.*, **13**, 8053–8063 (1985).
14) Petschek, J. P., Mermer, M. J., Scheckelhoff, M. R., Simone, A. A. and Vaughn, J. C. RNA editing in *Drosophila* 4f-rnp gene nuclear transcripts by multiple A-to-G conversions. *J. Mol. Biol.*, **259**, 885–890 (1996).
15) Petschek, J. P., Scheckelhoff, M. R., Mermer, M. J. and Vaughn, J. C. RNA editing and alternative splicing generate mRNA transcript diversity from the *Drosophila* 4f-rnp locus. *Gene*, **204**, 267–276 (1997).
16) Ma, K., Inglis, J. D., Sharkey, A., Bickmore, W. A., Hill, R. E., Prosser, E. J., Speed, R. M., Thomson, K. T., Jobling, M., Taylor, K., Wolfe, J., Cooke, H. J., Hargreave, T. B. and Chandley, A. C. A Y chromosome gene family with RNA-binding protein homology: candidates for the azoospermia factor AZF controlling human spermatogenesis. *Cell*, **75**, 1287–1295 (1993).
17) Mizrachi-Karsch, I. and Haynes, S. R. The Rb97D gene encodes a potential RNA-binding protein required for spermatogenesis in *Drosophila*. *Nucleic Acids Res.*, **21**, 2229–2235 (1993).
18) Baker, B. S. Sex in flies: the splice of life. *Nature*, **340**, 521–524 (1989).
19) Hodgkin, J. *Drosophila* sex determination: a cascade of regulated splicing. *Cell*, **56**, 905–906 (1989).
20) Kelly, R. L. Initial organization of the *Drosophila* dorsoventral axis depends on an RNA-binding protein encoded by the squid gene. *Genes Dev.*, **7**, 948–960 (1993).
21) Matunis, E. L., Kelly, R. and Dreyfuss, G. Essential role for a heterogeneous nuclear ribonucleoprotein (hnRNP) in oogenesis: hrp40 is absent from the germ line in dorsoventral mutant squid. *Proc. Natl. Acad. Sci. USA*, **91**, 2781–2784 (1994).
22) Robinow, S., Campos, A. R., Yao, K. M. and White, K. The elav gene product of *Drosophila*, required in neurons, has three RNP consensus motifs. *Science*, **242**, 1570–1572 (1988).
23) Chambers, J. C., Kenan, D., Martin, B. J. and Keene, J. D. Genomic structure and amino acid sequence domains of the human La autoantigen. *J. Biol. Chem.*, **263**, 18043–18051 (1988).
24) Deutscher, S. L., Harley, J. B. and Keene, J. D. Molecular analysis of the 60-kDa human Ro ribonucleoprotein. *Proc. Natl. Acad. Sci. USA*, **85**, 9479–9483 (1988).

25) Deutscher, S. L. and Keene, J. D. A sequence-specific conformational epitope on U1 RNA is recognized by a unique autoantibody. *Proc. Natl. Acad. Sci. USA*, **85**, 3299–3303 (1988).
26) Steiner, G., Hartmuth, K., Skriner, K., Maurer-Fogy, I., Sinski, A., Thalmann, E., Hassfeld, W., Barta, A. and Smolen, J. S. Purification and partial sequencing of the nuclear autoantigen RA33 shows that it is indistinguishable from the A2 protein of the heterogeneous nuclear ribonucleoprotein complex. *J. Clin. Invest.*, **90**, 1061–1066 (1992).
27) Szabo, A., Dalmanu, J., Manley, G., Rosenfeld, M., Wong, E., Henson, J., Posner, J. B. and Furneaux, H. M. HuD, a paraneoplastic encephalomyelitis antigen, contains RNA-binding domains and is homologous to Elav and Sex-lethal. *Cell*, **67**, 325–333 (1991).
28) Soulard, M., Della Valle, V., Siomi, M. C., Piñol-Roma, S., Codogno, P., Bauvy, C., Bellini, M., Lacroix, J. C., Monad, G., Dreyfuss, G. and Larsen, C. J. hnRNP G: sequence and chracterization of a glycosylated RNA-binding protein. *Nucleic Acids Res.*, **21**, 4210–4217 (1993).
29) Miyachi, K., Fritzler, M. J. and Tan, E. M. Autoantibody to a nuclear antigen in proliferating cells. *J. Immunol.*, **121**, 2228–2234 (1978).
30) Bravo, R., Frank, R., Blundell, P. A. and Macdonald-Bravo, H. Cyclin/PCNA is the auxiliary protein of DNA polymerase-δ. *Nature*, **326**, 515–517 (1987).
31) Chan, E. K., Imai, H., Hamel, J. C. and Tan, E. M. Human autoantibody to RNA polymerase I transcription factor hUBF. Molecular identity of nucleolus organizer region autoantigen NOR-90 and ribosomal RNA transcription upstream binding factor. *J. Exp. Med.*, **174**, 1239–1244 (1991).
32) Gottlieb, E. and Steitz, J. A. Function of the mammalian La protein: evidence for its action in transcription termination by RNA polymerase III. *EMBO J.*, **8**, 851–861 (1989).
33) Reimer, G., Rose, K. M., Scheer, U. and Tan, E. M. Autoantibody to RNA polymerase I in scleroderma sera. *J. Clin. Invest.*, **79**, 65–72 (1987).
34) Lerner, M. R. and Steitz, J. A. Antibodies to small nuclear RNAs complexed with proteins are produced by patients with systemic lupus erythematosus. *Proc. Natl. Acad. Sci. USA*, **76**, 5495–5497 (1979).
35) Moroi, Y., Peebles, C., Fritzler, M. J. and Tan, E. M. Autoantibody to centromere (kinetochore) in scleroderma sera. *Proc. Natl. Acad. Sci. USA*, **77**, 1627–1631 (1980).
36) Brenner, S., Pepper, D., Berne, M. E., Tan, E. M. and Brinkley, B. R. Kinetochore structure, duplication, and distribution in mammalian cells: analysis by human autoantibodies from scleroderma patients. *J. Cell Biol.*, **91**, 95–102 (1981).
37) Earnshaw, W. C., Sullivan, K. F., Machlin, P. S., Cooke, C. A., Kaiser, D. A., Pollard, T. D., Rothfield, N. F. and Cleveland, D. W. Molecular cloning of cDNA for CENP-B, the major human centromere autoantigen. *J. Cell Biol.*, **104**, 817–829 (1987).
38) Burnham, T. K. Antinuclear antibodies in patients with malignancies. *Lancet*, **ii**, 2436–2437 (1972).
39) Pupa, S. M., Menard, S., Andreola, S. and Colnaghi, M. Antibody response against the c-erbB-2 oncoprotein in breast carcinoma patients. *Cancer Res.*, **53**, 5864–5866 (1993).
40) Winter, S. F., Minna, J. D., Johnson, B. E., Takahashi, T., Gazder, F. and Carbone, D. P. 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).
41) Houghton, A. N. Cancer antigens: immune recognition of self and altered self. *J. Exp. Med.*, **180**, 1–4 (1994).
42) Sahin, U., Türeci, Ö., Schmitt, H., Cochlovius, B., Johannes, T., Schmits, R., Stenner, F., Luo, G., Schobert, I. and Pfreundschuh, M. Human neoplasms elicit multiple specific immune responses in the autologous host. *Proc. Natl. Acad. Sci. USA*, **92**, 11810–11813 (1995).
43) Old, L. J. and Chen, Y.-T. New paths in human cancer serology. *J. Exp. Med.*, **187**, 1163–1167 (1998).
44) Shichijo, S., Nakao, M., Imai, Y., Takasu, H., Kawamoto, M., Niiya, F., Yang, D., Toh, Y., Yamana, H. and Itoh, K. A gene encoding antigenic peptides of human squamous cell carcinoma recognized by cytotoxic T lymphocytes. *J. Exp. Med.*, **187**, 277–288 (1998).
45) Valenta, R., Natter, S., Seiberler, S., Wichlas, S., Maurer, D., Hess, M., Pavelka, M., Grote, M., Ferreira, F., Szepfalusi, Z., Valent, P. and Sting, G. Molecular characterization of an autoantigen, Hom s 1, identified by serum IgE from atopic dermatitis patients. *J. Invest. Dermatol.*, **111**, 1178–1183 (1998).
46) Corydon, M. J., Andresen, B. S., Bross, P., Kjeldsen, M., Andreasen, P. H., Eiberg, H., Kolvraa, S. and Gregersen, N. Structural organization of the human short-chain acyl-CoA dehydrogenase gene. *Mamm. Genome*, **8**, 922–926 (1997).
47) Schiffer, S. P., Prochazka, M., Jezyk, P. F., Roderick, T. H., Yudkoff, M. and Patterson, D. F. Organic aciduria and butyryl CoA dehydrogenase deficiency in BALB/cByJ mice. *Biochem. Genet.*, **27**, 47–58 (1989).
48) Bach, I., Galcheva-Gargova, Z., Mattei, M.-G., Simon-Chazottes, D., Guenet, J.-L., Cereghini, S. and Yaniv, M. Cloning of human hepatic nuclear factor 1 (HNF1) and chromosomal localization of its gene in man and mouse. *Genomics*, **8**, 155–164 (1990).
49) Turnbull, D. M., Bartlett, K., Stevens, D. L., Alberti, K. G. M. M., Gibson, G. J., Johnson, M. A., McCulloch, A. J. and Sherratt, H. S. A. Short-chain acyl-CoA dehydrogenase deficiency associated with a lipid-storage myopathy and secondary carnitine deficiency. *N. Engl. J. Med.*, **311**, 1232–1236 (1984).
50) Woo, S. L., Lidsky, A. S., Guttler, F., Chandra, T. and Robson, K. J. Cloned human phenylalanine hydroxylase gene allows prenatal diagnosis and carrier detection of classical phenylketonuria. *Nature*, **306**, 151–155 (1983).
51) Lidsky, A. S., Robson, K. J., Thirumalachary, C., Barker, P. E., Ruddle, F. H. and Woo, S. L. The PKU locus in man is on chromosome 12. *Am. J. Hum. Genet.*, **36**, 527–533

(1984).
52) Polymeropoulos, M. H., Ide, S. E., Magyari, T. and Francomano, C. A. Brachydactyly type C gene maps to human chromosome 12q24. *Genomics*, **38**, 45−50 (1996).
53) Jamieson, C. R., van der Burgt, I., Brady, A. F., van Reen, M., Elsawi, M. M., Hol, F., Jeffery, S., Patton, M. A. and Mariman, E. Mapping a gene for Noonan syndrome to the long arm of chromosome 12. *Nat. Genet.*, **8**, 357−360 (1994).
54) van der Vleuten, A. J. W., van Ravenswaaij-Arts, C. M. A., Frijns, C. J. M., Smits, A. P. T., Hageman, G., Padberg, G. W. and Kremer, H. Localization of the gene for a dominant congenital spinal muscular atrophy predominantly affecting the lower limbs to chromosome 12q23-24. *Eur. J. Hum. Genet.*, **6**, 376−382 (1998).
55) Timmerman, V., De Jonghe, P., Simokovic, S., Lofgren, A., Beuten, J., Nelis, E., Ceuterick, C., Martin, J. J. and Van Broeckhoven, C. Distal hereditary motor neuropathy type II (distal HMN II): mapping of a locus to chromosome 12q24. *Hum. Mol. Genet.*, **5**, 1065−1069 (1996).
56) Isozumi, K., DeLong, R., Kaplan, J., Deng, H.-X., Iqbal, Z., Hung, W.-Y., Wilhelmsen, K. C., Hentati, A., Pericak-Vance, M. A. and Siddique, T. Linkage of scapuloperoneal spinal muscular atrophy to chromosome 12q24.1-q24.31. *Hum. Mol. Genet.*, **5**, 1377−1382 (1996).
57) Mitelman, F., Mertens, F. and Johansson, B. A breakpoint map of recurrent chromosomal rearrangements in human neoplasia. *Nat. Genet.*, **15**, 417−474 (1997).
58) Marchand, M., van Baren, N., Weynants, P., Brichard, V., Dréno, B., Tessier, M.-H., Rankin, E., Parmiani, G., Arienti, F., Humblet, Y., Bourlond, A., Vanwijck, R., Liénard, D., Beauduin, M., Dietrich, P.-Y., Russo, V., Kerger, J., Masucci, G., Jäger, E., de Greve, J., Atzpodien, J., Brasseur, F., Coulie, P. G., van der Bruggen, P. and Boon, T. Tumor regressions observed in patients with metastatic melanoma treated with an antigenic peptide encoded by gene MAGE-3 and presented by HLA-A1. *Int. J. Cancer*, **80**, 219−230 (1999).
59) Ibe, M., Moore, Y. I., Miwa, K., Kaneko, Y., Yokota, S. and Takiguchi, M. Role of strong anchor residues in the effective binding of 10-mer and 11-mer peptides to HLA-A2402 molecules. *Immunogenetics*, **44**, 233−241 (1996).