

Molecular Cloning of Canine RCAS1 cDNA

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ABSTRACT. Receptor-binding cancer antigen expressed on SiSo cells (RCAS1) is a novel cancer cell-surface antigen, strongly expressed in invasive cancers. RCAS1 inhibited the *in vitro* growth of immunocytes, and induced apoptotic cell death. The cloning of canine RCAS1 cDNA was carried out and identified from the mammary gland tumor of a dog. A canine RCAS1 cDNA of 864 bp in length has an open reading frame of 642 bp nucleotides encoding a protein of 213 deduced amino acids. The predicted amino acid sequence of canine RCAS1 showed 96.2% and 96.7% homologies with those of human and mouse RCAS1 respectively. Canine RCAS1 has an N-terminal transmembrane segment and a coiled-coil structure in the C-terminal protein, which are highly conserved in mouse and human RCAS1.

KEY WORDS: canine, cDNA, RCAS1.

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Receptor-binding cancer antigen expressed on SiSo cells (RCAS1) has been recognized as a novel tumor-associated antigen in a human uterine cervical adenocarcinoma cell line using the monoclonal antibody 22-1-1 [14]. RCAS1 was identified with the estrogen receptor-binding fragment-associated gene 9 (EBAG9), which was recognized as an estrogen-responsive gene from a cDNA library of MCF-7 human breast cancer cells [18]. Immunohistochemical studies have shown that RCAS1 is expressed in uterine and ovarian carcinomas. RCAS1 is expressed in the cytoplasm, cell membrane and glandular lumen of adenocarcinoma cells, and its expression especially strong in invasive carcinomas [12, 14, 15]. However, RCAS1 is not found in normal uterine cervix tissue or squamous dysplasias [13]. RCAS1 has been detected not only in adenocarcinoma but also breast carcinoma, gastric cancer, hepatocellular carcinoma, lung cancer, pituitary adenoma and esophageal squamous cell carcinoma [3, 6, 7, 9, 16, 17]. Expression of RCAS1 indicates a poor prognosis in patients with many kinds of cancer [3-7, 11-16].

RCAS1 acts as a ligand for a putative receptor present on hematopoietic cells and normal peripheral lymphocytes such as T, B and NK cells. RCAS1 inhibits the growth of receptor expressing cells *in vitro* and induces apoptotic cell death. Therefore, probably RCAS1 plays a role in the escape of tumor cells from immune surveillance [8], and also may act as a failsafe mechanism to inhibit maternal immune attack and maintain pregnancy [10].

In veterinary practice, it is important to know the prognosis of various tumors in dogs. In order to acquire immunological tools clarify the role of canine RCAS1 in escape from immune surveillance, it is necessary to study the cDNA for canine RCAS1. The RCAS1 gene has been

cloned in humans and mice, but little is still known about canine RCAS1 cDNA. Therefore, the aim of the present study was to clarify the cDNA nucleotide and amino acid sequences of canine RCAS1.

Total RNA was extracted from a surgically resected sample of a canine mammary carcinoma (complex carcinoma) using an RNeasy Mini kit (Quiagen, Hilden, Germany) according to the instructions of the manufacturer. Reverse transcription of the RNA was performed with a Sensiscript RT kit (Quiagen) using a 38 bases oligonucleotide primer (AP) with 17 dT bases, followed by an adaptor sequence as 5'-GGCCACGCGTCTGACTAC-3'. Oligonucleotide primers were designed on the basis of the sequences of the human and mouse RCAS1 cDNAs (Table 1). TITANIUM Taq DNA polymerase (Clontech, CA, U.S.A.) was used in PCR for amplification of the canine RCAS1 cDNA. To amplify the 3' end and 5' end of cDNA encoding the canine RCAS1, rapid amplification of 3' cDNA ends (3' RACE) and 5' cDNA ends (5' RACE) were performed using a 3' RACE System Kit (GibcoBRL, MD, U.S.A.) and 5' RACE System Kit version 2.0 (GibcoBRL). The PCR products were cloned in the pCR2.1 vector (Invitrogen Corporation, CA, U.S.A.), subjected to sequence analysis by using Termo Sequenase Pre-mixed Cycle Sequencing Kit (Amersham Biosciences, NJ, U.S.A.) and read by a HITACHI SQ-5500 DNA sequencer.

Canine RCAS1 cDNA is 864 bp (GenBank accession number: AB083366) in length, and has an open reading frame of 642 nucleotides encoding a protein of 213 amino acids, comprising a signal peptide sequence of 27 amino acids (Fig. 1). The ATG initiation codon is in a standard Kozak consensus sequence (CCCACCATG, position number -6~3 of canine RCAS1 cDNA). Canine RCAS1 has an N-terminal transmembrane segment (amino acids 8-27), as predicted by Tmbase [2], and a coiled-coil structure in the C-terminal portion of the protein (amino acids 163-203), as

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Table 1. Sequences of oligonucleotide primers

| Primers | Primer sequences | Purpose |
|---------|---|--|
| S1 | 5'-ATGGCCATCAC(A/C/G/T)CAGTTTC GGTTAT-3' | For amplifying a part of canine RCAS1 cDNA |
| AS2 | 5'-TCTTCTGCTTGCCAGGCTGCATCTT-3' | |
| 3'-1 | 5'-TCCAGATGGTAGCACAGGTTTCTCT-3' | For amplifying 3' cDNA end |
| 3'-2 | 5'-TATGCCTTTTATTCATCAATCTCCT-3' | Nasted primer for 3' RACE |
| 5'-1 | 5'-TCCCATTCCTCCTTCAATCTTTAC-3' | For amplifying 5' cDNA end |
| 5'-2 | 5'-GGAAGTCCACTCTTCAACATCAGTCT-3' | Nasted primer for 5' RACE |
| rR-SP | 5'-GAATTCATGGCTATCACACAGTTTCG-3' | For amplifying coding region of canine RCAS1 cDNA |
| rR-AP | 5'-CTCGAGTTATGAAAGCTTCACGCC-3' | |

Oligonucleotide primers, S1 and AS2, were designed based on the sequences conserved between human and mouse RCAS1 cDNAs.

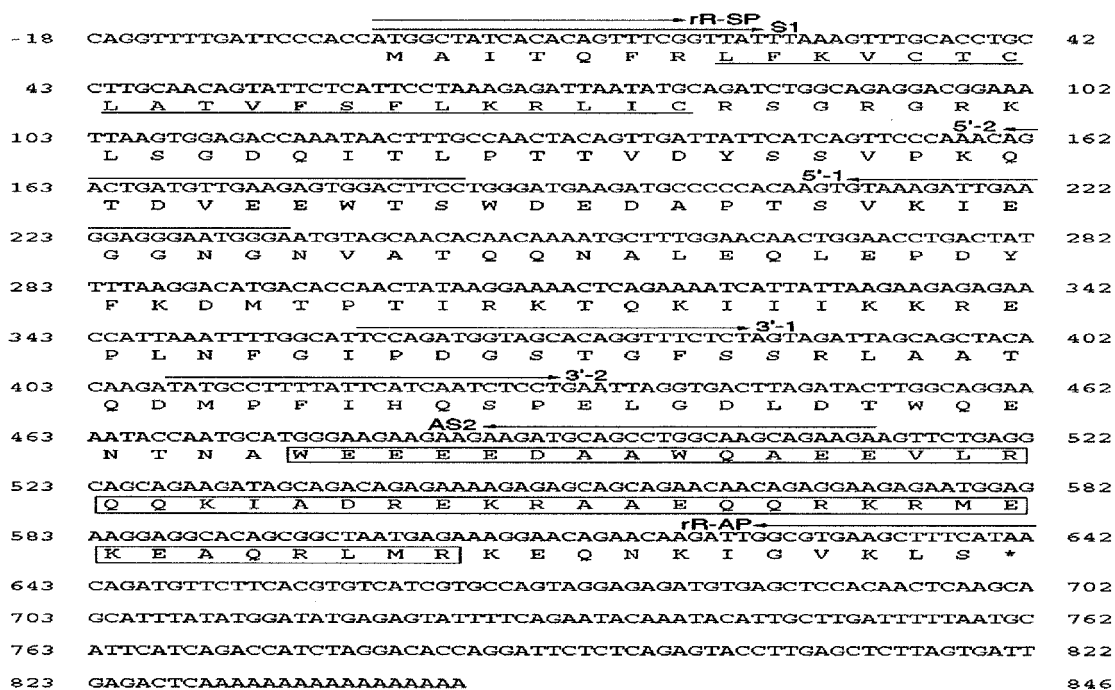


Fig. 1. Nucleotide and deduced amino acid sequences of canine RCAS1 (GenBank accession number: AB083366). Asterisk is positioned to show the stop codon. Underlined sequences are predicted to transmembrane helices. A predicted coiled-coil structure is boxed. The arrows indicate the sequences of oligonucleotide primers.

predicted by Paircoil [1], indicating that canine RCAS1 is a type II membrane protein able to form oligomers through the coiled-coil structure.

The predicted amino acid sequence of canine RCAS1 and multiple alignments with its counterparts from other species were generated by a genetic information processing software package, GENETYX-MAC Ver. 9.0 (Software development, Tokyo, Japan). Canine RCAS1 cDNA shows high homologies with its human (91.7%) and mouse (89.9%) counterparts, and the predicted amino acid sequence of the protein showed 96.2% and 96.7% homologies with human and mouse RCAS1, respectively (Fig. 2).

It is concluded that canine RCAS1 cDNA has a coding region of 642 nucleotides encoding a protein of 213 amino

acids. RCAS1 is highly conserved between human, mouse and canine in the amino acid level, suggesting that RCAS1 has biologically essential function beyond the species. The availability of canine RCAS1 cDNA will provide a useful reagent to examine on its expression patterns, synthesize recombinant protein and produce monoclonal antibodies of canine RCAS1. Immunohistochemical studies has shown that high expression of human RCAS1 is a poor prognostic factor in uterine, ovarian, breast and hepatocellular carcinomas, gastric cancer, lung cancer, pituitary adenoma and esophageal squamous cell carcinoma [3, 6, 7, 9, 12, 14–17]. Recombinant human RCAS1 inhibits the proliferation and induced DNA fragmentation of human peripheral blood lymphocytes [8]. Therefore, the synthesis of recombinant

| | | | |
|--------|-----|---|-----|
| Canine | 1 | MAITQFRLFKVCTCLATVFSFLKRLICRSGRGRKLSGDQITLPTTVDYSSVPKQTDVEEW | 60 |
| Human | 1 | *****F***** | 60 |
| Mouse | 1 | ***** | 60 |
| | | | |
| Canine | 61 | TSWDEDAPTSVKIEGGNCNVATQQNALEQLEPDYFKDMTPTIRKTKQKIIKKREPLNFGI | 120 |
| Human | 61 | *****S*****V***** | 120 |
| Mouse | 61 | *****S*****V*****V | 120 |
| | | | |
| Canine | 121 | PDGSTGFSSRLAATQDMPFIHQSPELGDLDTWQENTNAWEEEEEEDAANQAEVLRQOKIAD | 180 |
| Human | 121 | *****I*****S*****L** | 180 |
| Mouse | 121 | *****S*****S***** | 180 |
| | | | |
| Canine | 181 | REKRAABQQRKRMEKEAQRIMRKEQNKIGVKLS | 213 |
| Human | 181 | *****K*****K***** | 213 |
| Mouse | 181 | *****K*****K***** | 213 |

Fig. 2. Comparison of amino acid sequence of canine RCAS1 with those of human RCAS1 (GenBank accession number: AF006265) and mouse RCAS1 (GenBank accession number: AF076524). Asterisks indicate identical amino acids.

canine RCAS1 and the production of monoclonal antibodies against it will be extremely useful for research on canine oncology and apoptotic cell death.

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