Properties of the Tumor Suppressor Gene Brca2 in the Cat

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Abstract. Mammary tumors are common in cats. As mutations in human Brca2 confer an increased risk of breast cancer, the full-length cDNA of the feline homologue of Brca2 was sequenced to obtain a basis for studying the relationship between its function and susceptibility to mammary tumors. The feline Brca2 cDNA is 10 kb long, and encodes 3,371 amino acids. The amino acid sequence of feline Brca2 shares low homology with the Brca2 of other mammals, e.g., 53% homology with the murine protein. Analysis of the expression pattern of the feline Brca2 gene revealed that, as previously reported for other mammals, it is transcribed in various tissues, including the mammary gland.

Key words: Brca2, feline, mammary tumor.

Breast tumors are more common in the female cat than in any other domestic species except the dog [6], and account for 17% of the neoplasms in female cats [17]. The percentage of breast tumors that are malignant is higher in the cat (86%) than in the dog (42%). Adenocarcinoma predominates among malignant mammary tumors in cats [11], and in many biological and histological aspects (e.g., rapid growth, high metastatic rates to distant organs at an early stage, and poor prognosis) it resembles adenocarcinoma in women more closely than does experimentally induced cancer in inbred laboratory animals [10, 24]. In both humans and cats, treatment of advanced breast cancer is often futile and disfiguring, making early detection a high priority in the management of this disease.

Germline mutation of the Brca2 gene in humans carries a high risk of developing breast cancer [16]. The Brca2 gene appears to act as a tumor-suppressor gene, in that carriers show loss or mutation of the wild-type allele within tumors (loss of heterozygosity). After sequencing the full-length cDNA, many reports have described mutational analyses of the gene in families and in primary tumors [9, 12, 21]. The Brca2 cDNA sequence, which predicts a large protein of 3,418 amino acids, shows no obvious homology to any other protein [5, 26]. The tumor suppressor function of Brca2 appears to be associated with its biochemical interaction with Rad51 protein, which plays a central role in maintaining chromosome stability and double-strand break repair [1, 14, 19, 25].

To establish a basis for diagnostic methods and for using as a prognostic factor, and to understand the role of Brca2 in mammary tumors, we report the sequence of feline Brca2 cDNA. We also studied the pattern of gene expression.

The polymerase chain reaction (PCR) and direct sequencing were performed using the primers shown in Table 1. The amplified fragments are shown in Fig. 1A. The primer sequences were designed from reported human (GenBank accession No.: NM_000059), murine (U65594), and canine (AB043895) sequences, or designed from the feline sequence that we obtained using these primers. Total RNA was extracted from the testis of a mongrel male cat using TRIzol reagent (Invitrogen, Leek, The Netherlands). Total RNA (2 µg) was denatured at 70°C for 10 min, cooled immediately, and reverse transcribed using 200 units of M-MLV reverse transcriptase (Invitrogen), 25 pmol of random primer, and 10 nmol dNTPs in a total volume of 20 µl at 37°C for 50 min. After heating at 75°C for 15 min, PCR amplification was performed with 2.5 units of Taq polymerase or KOD Plus (TOYOBO, Osaka, Japan), 1.5 mM MgCl2, 20 nmol of dNTPs, and 20 pmol of primers. PCR was conducted for 30 cycles, each consisting of denaturation at 94°C for 30 sec, annealing at 53°C for 30 sec, and extension at 68°C for 2.5 min. PCR amplification of the 5’ and 3’ cDNA ends of feline Brca2 was performed with the RACE system (Invitrogen), and 10 pmol of the target-specific primers shown in Fig. 1. For the RACE system, PCR was conducted for 30 cycles, each consisting of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec, and extension at 72°C for 5 min. The sequences were determined by direct sequencing using BigDye Terminator Cycle sequencing reagents and an Applied Biosystem Sequencer 310 (Perkin Elmer, Tokyo, Japan).

To explore the tissue distribution of feline Brca2, the RT-reaction was performed for total RNA extracted from testis, prostate, ovary, mammary gland, spleen, thymus, lymph node, heart, liver, kidney, and brain. PCR amplification was performed with the primers shown in Fig. 1, with forward primer No. 8 and reverse primer No. 9. RT-PCR was performed as described for cDNA sequencing, except that 1 µg of total RNA was used. The PCR products were electrophoresed on 1% agarose gels and stained with ethidium bromide. Glyceraldehyde-phosphate dehydrogenase (GPDH) was used as a control.

The open reading frame of the feline Brca2 cDNA was 10 kb long, encoding a large protein of 3,371 amino acids (accession No. AB107955). The feline Brca2 protein...
showed 68, 53, 78, and 32% homology with the human [21],
murine [18], canine [15], and chicken Brca2 proteins [20,
23], respectively. The degree of homology for feline and
other mammals is low, but similar to values reported previ-
ously for mouse and human Brca2 proteins [18]. Thus, the
degree of homology is sufficient to deem that the PCR prod-
uct obtained in this study is feline Brca2. In fact, we have
not produced any other homologous PCR products using our
PCR procedure. Feline Brca2 includes predicted functional
domains, such as BRC repeats [3], P/CAF interacting motif
[8], three of NLSs [27], BLAT domain [23], and C-terminus
Rad51 binding domain [14, 19], that are conserved in the
species reported to date (Fig. 1B). The tissue distribution of
feline Brca2 was investigated by RT-PCR, using RNA from
brain, lymph node, thymus, heart, liver, kidney, testis, mam-
mary gland, prostate, ovary, and uterus (Fig. 2). As previ-
ously reported for the human and mouse, Brca2 was ex-
pressed in the mammary gland, testis, ovary, uterus, thy-
mus, and brain. Because the functional domains are impor-
tant for the tumor suppressor function of human Brca2 [4, 8,
23, 27], and because feline Brca2 was expressed in mam-
mary gland, it may be possible that feline Brca2 also acts as
tumor suppressor in the cat.

Since the human Brca2 mutations that cause mammary
tumors occur throughout its sequence [22], it was deemed
necessary to obtain the entire feline Brca2 sequence. There-
fore, we amplified the full-length feline Brca2 cDNA by
RT-PCR. This should permit the genetic analysis of feline
breast cancer, to determine whether mutations of this gene
are also responsible for a predisposition to mammary tumors
in felines. If so, this technique will be useful for genetic
diagnosis of hereditary mammary tumors in the cat. Since it
has been reported that some feline breeds have twice the risk
of developing mammary carcinoma as all breeds combined
[11], the presence of a mammary tumor susceptibility gene
in the cat, such as Brca2, may be possible. Loss of Brca2
expression in the cat may cause tumors, since the human
homologue is established as a tumor suppressor. Con-
versely, overexpression of Brca2 in human sporadic breast
cancer is reported to predict a poor prognosis [2, 7]. In addi-
tion to several proposed prognostic factors for feline mam-
mary tumors [13], such as the age of onset and the diameter

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<th>Table 1. Profiles of the PCR products and primers used in PCR and sequencing of feline Brca2</th>
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For the RACE fragments (fragments I and XII) only the specific primers are shown. Under lined sequences are
obtained from feline Brca2 sequence.
of the primary tumor, feline Brca2 mRNA expression might also be a prognostic factor in feline mammary tumors.

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