Characterization of Alkaline Phosphatase Genes Expressed in Seminoma by cDNA Cloning

Atsuko Shigenari, Asako Ando, Tamami Baba, Tateki Yamamoto, Yoji Katsuoka, and Hidetoshi Inoko

Department of Molecular Life Science, Tokai University School of Medicine, Kanagawa 259-1193 [A. S., A. A., T. B., H. I.]; Natural Products Research Department, Teikoku Hormon Manufacturing Co., Ltd., Kawasaki 213-0033 [T. Y.]; and Department of Urology, Osaka Medical College, Osaka 569 [Y. K.]. Japan

Abstract

Two members of a placental alkaline phosphatase (PLAP) family, PLAP and PLAP-like or germ cell alkaline phosphatase, are aberrantly expressed in tumors of ectopic origin. To characterize alkaline phosphatase induced in seminoma, alkaline phosphatase cDNA clones were isolated from a cDNA library constructed from seminoma cells and characterized by nucleotide sequence determination. Thus, isolated cDNA clones were classified into two types, germ cell alkaline phosphatase (PLAP-like) and liver/bone/kidney-type alkaline phosphatase (L/B/K AP). These results suggest that other than the PLAP family members, the expression of L/B/K AP is enhanced in seminoma and can serve as a tumor marker in seminoma.

Introduction

Human APs can be classified into at least five tissue-specific forms or isozymes mainly according to the specificity of the tissue to be expressed, termed PLAP (or Regan isozyme), IAP, L/B/K AP, germ cell AP (PLAP-like or NAGAO isozyme), and IAP-like (Kasahara isozyme; Ref. 1). AP is present in nearly all living organisms, with the exception of some higher plants, but little is known regarding the physiological function of AP in most tissues, except that the bone isozyme (L/B/K AP) is supposed to have a role in normal skeletal mineralization (2); a missense mutation in the L/B/K AP gene is responsible for the development of hypophosphatasia (3).

The physiological function of AP in most tissues, except that the bone isozyme (L/B/K AP) is supposed to have a role in normal skeletal mineralization (2); a missense mutation in the L/B/K AP gene is responsible for the development of hypophosphatasia (3).

Materials and Methods

Construction of a Seminoma cDNA Library. A seminoma type of testicular cancer cells at stage IIB was obtained from a 24-year-old Japanese man. Total RNA was extracted from them by the homogenate procedure in the guanidine thiocyanate solution followed by CsCl centrifugation at 36,000 rpm and 20°C for 12 h. Poly(A)-enriched RNA was purified by oligo-deoxynucleotide cellulose chromatography. A seminoma cDNA library was constructed from this poly(A)-enriched RNA using the ZAP-cDNA synthesis kit according to the protocol provided by the manufacturer (Stratagene, La Jolla, CA). Briefly, the first-strand cDNA was synthesized by priming of Moloney murine leukemia virus reverse transcriptase reactions with oligo(dt) linker primer including a Xhol recognition site using 5-methyl dCTP, which protects the cDNA from restriction enzyme used in subsequent cloning step. The product of first-strand synthesis, a cDNA-mRNA hybrid, was used as a template for nick-translation reaction with RNase H. This reaction created a series of RNA primers that was used by Escherichia coli DNA polymerase I to produce the second strand of cDNA. The uneven termini of the resultant cDNA species were filled in with Pfu polymerase, and EcoRI adapters were ligated to the blunt ends. Then, the Xhol-digested and size-fractionated cDNA was ligated to the Uni-ZAP XR vector arms at the Xhol-EcoRI sites. This library consisted of ~1 × 10^6 independent recombinant phages.

Probe Screening of a cDNA Library. Two PCR-amplified products containing portions of the germ cell AP gene (PLAP-like) were used for screening of the cDNA library as probes. A 1051-bp fragment encompassing the segment from the midst of exon 3 to exon 6 in the germ cell AP gene (from nucleotide positions 903 to 1953; Ref. 14) was amplified using the following primers: primer 1 (forward), 5'-CCGCTGAGACCTCTGATGGA-3' and primer 2 (reverse), 5'-CTGCTGCTGTGACGCTGCAAAGT-3'. Another 653-bp fragment encompassing the segment from the midst of exon 9 to the midst of exon 11 (from nucleotide positions 2616 to 3268; Ref. 14) was amplified using the following primers: primer 3 (forward), 5'-GCGGCTTGCTGCCAGCCACTTC-3' and primer 4 (reverse), 5'-GAAAGCCTAGCAGGGCCCTTC-3'. PCR reactions were carried out using 100–200 ng of high molecular mass human genomic DNA of Japanese origin with the 5 mM PCR primer pair in a standard 25-ml reaction containing 1.2 units of TAKARA LA Taq polymerase (TakaraBio, Ohtsu, Shiga, Japan), 0.2 mM deoxynucleotide triphosphate, 25 mM MgCl2, and LA PCR buffer (Takara) Reaction mixture was placed in a

Received 7/27/98; accepted 10/4/98.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 To whom requests for reprints should be addressed, at Department of Molecular Life Science, Tokai University School of Medicine, B2, Seaside, Isehara, Kanagawa 259-11, Japan. Phone: 81-463-93-1121 (extension 2312); Fax: 81-463-94-8884.

2 The abbreviations used are: AP, alkaline phosphatase; PLAP, placental AP; L/B/K AP, liver/bone/kidney-type AP; IAP, intestinal AP.

3 T. Yamamoto and Y. Katsuoka, unpublished data.
ALKALINE PHOSPHATASE IN SEMINOMA

Results and Discussion

To isolate cDNA clones encoding APs induced in seminoma, a cDNA library was constructed from poly(A)-enriched RNA prepared from a seminoma type of testicular cancer cells. This cDNA library was screened by plaque hybridization with the two genomic probes, with or without overlap with the germ cell AP gene (14). Furthermore, the deduced amino acid sequence of p5-2 reveals 98.4% amino acid identity in a 125-amino-acid overlap with germ cell AP. There are only two amino acid substitutions, Glu for Gly at residue 434 and Ser for Pro at Thr at residue 531 between p5-2 and another variant of germ cell AP reported previously by Gum et al. (9). A putative poly(A) signal AATAAA is underlined. This cDNA clone represents a transcript from a new allele of the germ cell AP gene.

Fig. 1. Nucleotide and deduced amino acid sequences of the cDNA clones derived from seminoma, p5-2 encoding AP. Arrows, 22 nucleotide substitutions between p5-2 and one variant of germ cell AP reported previously by Millan and Manes (14). *, two amino acid substitutions, Glu for Gly at residue 434 and Ala for Thr at residue 531 between p5-2 and another variant of germ cell AP reported previously by Gum et al. (9). A putative poly(A) signal AATAAA is underlined. This cDNA clone represents a transcript from a new allele of the germ cell AP gene.

segments, which contain the residues that interact with substrate phosphate (exons 4 and 5) and metal ligand sites (exons 5 and 9), are well conserved among the five AP genes (PLAP, IAP, L/B/K AP, PLAP-like, and IAP-like) thus far cloned. The exon 9–11 segment of the germ cell AP gene displays more than 98 and 90% identity at the nucleotide level with the PLAP and IAP genes, and more than 70% with the most distantly related gene, L/B/K AP. Such high nucleotide identity in these segments possibly allows the probes used for screening of the library to pick up cDNA clones for any type of the APs, if they were expressed in seminoma.

In total, eight cDNA clones among 1 × 10^5 plaques were thus isolated and constructed for their restriction maps using representative six-base recognition restriction enzymes. Although any of them did not seem to represent full-length transcripts, these cDNA clones could be classified into two groups (three and five clones) in this way. Two clones, p5-2 and p5-12, carrying the longest inserts were chosen among the former and latter groups, respectively, and subjected to nucleotide sequence determination.

The cDNA insert of p5-2 consists of 1234 bp, including most of the one-third of the transcript, 375 bp of the 3' terminal coding region (125 residues from the COOH-terminal end), and the entire 3' untranslated region (Fig. 1). The p5-2 cDNA nucleotide sequence reveals 98.2% identity (22 nucleotide differences) in a 1230 nucleotide translated region (Fig. 1). The p5-2 cDNA nucleotide sequence was determined.

*The nucleotide sequence data reported in this study will appear in DNA Data Bank of Japan, European Molecular Biology Laboratory and GenBank nucleotide sequence databases with the following accession numbers: AB012642 and AB012643.
Fig. 2. Nucleotide and deduced amino acid sequences of the cDNA clones derived from seminoma. p15-2 encoding AP. A putative poly(A) additional signal AATAAA is underlined. This cDNA clone represents a transcript from the L/B/K AP gene*, translational termination codon.

substitutions, Glu for Gly at residue 434 and Ala for Thr at residue 531 (Fig. 1). These results suggest that p5-2 represents a transcript from a new allele of the germ cell AP gene. The cDNA insert of the other clone, p15-2, consists of 1799 bp including the almost two-thirds of the transcript, 1023 bp of the 3'-terminal coding region (341 residues from the COOH-terminal end) and the entire 3' untranslated region (Fig. 2). The p15-2 cDNA nucleotide sequence reveals complete identity in a 1390-nucleotide overlap with the L/B/K AP cDNA clone reported by Kishi et al. (16), although their cDNA clone is apparently lacking the most 3' end segment, including a poly(A) tail. p15-2 also shows high nucleotide identity (98.5%) in a 1033-nucleotide overlap with another L/B/K AP cDNA clone with a different allele (7). Between them, there is only one amino acid substitution, His for Tyr at residue 263. These results clearly demonstrate that p15-2 represents a transcript from the L/B/K AP gene.

Despite an apparent association between the induction of AP and various cancers (10–13), no distinct differences between testicular tumor-derived AP and thus far known APs such as PLAP have been recognized (14, 15). In this study, eight cDNA clones for AP were isolated from the seminoma cDNA library. Among them, three and five clones represent transcripts from the PLAP-like and L/B/K AP genes, respectively. The number of the cDNA clones isolated and characterized in this study was limited, and these cDNA analyses were qualitative rather than quantitative. However, these results suggest that germ cell AP and L/B/K AP are two major species strongly reexpressed in seminoma. In addition, both of germ cell AP and L/B/K AP could be detected in seminoma by biochemical analyses such as calibration of molecular weight and enzyme assay in the presence of various inhibitors, confirming the expression of these two PLAP-like and L/B/K AP genes in seminoma. To our knowledge, our study here is the first finding of up-regulation of L/B/K AP in seminoma.
seminoma. Thus, the expression of L/B/K AP as well as germ cell AP can serve as a tumor marker in seminoma. The expression of L/B/K AP enzyme activity is under the control of various stimuli at the transcriptional level (17–19). In particular, two different mRNAs produced by alternative splicing or alternative transcription initiation in the mouse or rat, respectively, appear to be strictly regulated for the tissue-specific expression in response to several hormones and other regulators (19, 20). The induction of L/B/K AP associated with tumorization such as in seminoma might result from the involvement of the same regulatory factors controlling such expression of L/B/K AP enzyme activity in various tissues, which remains to be determined.

References

Characterization of Alkaline Phosphatase Genes Expressed in Seminoma by cDNA Cloning

Atsuko Shigenari, Asako Ando, Tamami Baba, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/58/22/5079

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.