Subtractive Cloning of a Hybrid Human Endogenous Retrovirus and Calbindin Gene in the Prostate Cell Line PC3

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Abstract

A complementary DNA clone containing human endogenous retrovirus-like sequences spliced to sequences encoding human calbindin was discovered by complementary DNA subtraction analysis between two human prostate cell lines, PC3 and DU145. This gene is presumably activated by the long terminal repeat of the retrovirus-like sequence. It belongs to a member of the retrovirus-like H (tRNA3-primer binding sequence) family. The level of this transcript is high in PC3, derived from a prostate bone metastasis, but not in DU145, derived from a prostate brain metastasis.

Introduction

Many prostate cancers metastasize to bone and, if left unchecked, more than 80% of patients with prostate cancer would develop bone metastases. Metastases to other organs are detected less frequently (1). To study prostate cancer metastasis, we used the technique of subtractive cDNA cloning (2) to identify genes that may play a role in this process. We carried out a subtraction between the cDNA libraries of two prostate cell lines established from metastases, PC3 (3) and DU145 (4). PC3 originated from the hormone-insensitive prostate cancer of a 62-year-old patient and was established from a vertebral body metastasis composed of poorly differentiated tumor cells. DU145 originated from the prostate cancer of a 69-year-old patient and was established from a brain metastasis composed of moderately to poorly differentiated tumor cells. Both PC3 and DU145 are aneuploid and tumorigenic; PC3, in addition, exhibits metastasis in nude mice (5). Other than the fact that these two cell lines behave somewhat differently in nude mice the reason behind the experiment is to determine the molecular lesions in these cells. It has been discovered that DU145, unlike PC3, expresses a mutant retinoblastoma gene, and its tumorigenicity can be suppressed by the normal retinoblastoma gene (6).

Materials and Methods

Cell Lines. PC3 and DU145 were obtained from the ATCC (Rockville, MD). PC3 (CRL 1435) was cultured in Ham’s F-12K medium-10% FBS. DU145 (HTB 81) was cultured in Eagle’s minimal essential medium-10% FBS. Nonprostatic cell lines CEM (ATCC CCL 119, acute lymphoblastic leukemia) and K562 (ATCC CCL 243, chronic myelogenous leukemia) were cultured in RPMI 1640-10% FBS.

Commercially available M13 sequencing 24-mer (AGCGGATACCAATTTCCACAGGA). Transformation into bacteria was done by electroporation [Gene Pulser and Pulse Controller (Bio-Rad, Richmond, CA) with these settings: 2.5 kV, 25 μF, 200 Ω.] (11). From 15 to 20% of clear colonies of the [PC3 minus DU145] library or the [DU145 minus PC3] library contained recombinant clones with cDNA inserts ranging in size from 400 to 1800 base pairs. RNA dot blots were used to test the cell specificity of candidate clones. 0.1 μg Poly(A)* RNA of PC3 and DU145 were applied separately onto filters. The clones were scored as ones that showed hybridization to only one RNA source.

DNA Sequence Analysis. cDNA clones were subcloned into M13mp18 or M13mp19. DNA sequencing was performed with the USB Sequenase kit, version 2.0 (United States Biochemical, Cleveland, OH).

Results and Discussion

Poly(A)* RNA was isolated from PC3 and DU145; cDNA libraries were constructed in pLOT731. In subtraction [PC3 minus DU145], the DU145 SSCDNA was biotinylated, and a 10-fold excess of the biotin-cDNA was hybridized to PC3 SSCDNA. After hybridization, all common cDNA was in duplex form and, together with the unhybridized DU145 cDNA, was separated from the nonhybridized (nonbiotinylated) PC3 cDNA by the addition of streptavidin and extraction with phenol. Biotinylated molecules were partitioned into the organic phase. The subtracted cDNA present in the aqueous phase was transformed into bacteria to form the subtraction libraries. In subtraction [DU145 minus PC3], the same process was repeated with biotinylated PC3 SSCDNA.

We analyzed 14 candidate PC3-specific clones from the [PC3 minus DU145] library and 21 candidate DU145-specific clones...
Fig. 1. Identification of P25 as a PC3-specific cDNA. Equivalent amounts of poly(A)* RNA of PC3 and DU145 were probed on filters by the various cDNAs. The cDNA inserts were labeled and added to the filter pieces. Clones P25 and P58 showed hybridization to PC3 RNA only; hence they are PC3 (not DU145) specific. Clones P61, P69, and P83 showed hybridization to both RNAs and are therefore common cDNAs. Note the equal signal intensity in either RNA dot for each probe and the intensity difference among the three, an indication of the abundance class to which each of these genes belongs. P25 was found in two other human cell lines, K562 and CEM, poly(A)* RNA of which were available; however, we cannot conclude whether this is due to calbindin or RTVL sequences. Arrow indicates the DU145 spot.

Fig. 2. The 5' sequence of clone P25. The autoradiogram (A) shows the junction sequence between RTVL-H and calbindin. B, hypothetical translated sequence of P25. *, border of the LTR and the primer binding sequence (PBS), †, splice junction of RTVL and calbindin. EL-LL--- motif, functional EF-hand of calcium binding proteins, where E represents glu, L, any hydrophobic residue, --, any residue, *, oxygen atom containing residue, G, gly.

from the [DU145 minus PC3] library. The specificity of these clones was tested by dot-blot hybridization with poly(A)* RNA isolated from PC3 and DU145 (see Fig. 1). PC3-specific clones should show hybridization to PC3 RNA only; DU145-specific ones showed hybridization to DU145 RNA only. We identified three PC3-specific clones: P25 (with a 1.5-kilobase insert), P58 (0.5-kilobase insert), and P85 (1.4-kilobase insert); but no DU145-specific ones.

The DNA sequences of P58 and P85 do not match any known sequences; that of P25 shows that it encodes the gene for human calbindin D28K, a cytosolic 28-kDa calcium-binding protein (13). However, at the 5' terminus a noncalbindin sequence was found. This noncalbindin sequence is 93% similar to that located downstream of the LTR promoter region of RTVL-H (14). There are about 1000 copies of RTVL-H in the human genome. In P25, the RTVL-H sequence replaces the 5' terminus of the calbindin gene. The junction of the two sequences is located precisely at an RNA splice joint (Fig. 2). It appears that RNA splicing has taken place between the first splice donor downstream of the LTR and the splice acceptor of the second exon of calbindin. A match of 30 nucleotides was found between the P25 RTVL sequence and that encoding the first 10 amino acid residues of an open reading frame of a cDNA clone, cTP4, isolated from a placenta library. cTP4 was identified as a transcript derived from RTVL-H3 (15). Given the structure of P25, is it now clear that the open reading frame of cTP4 was generated from a splice in RTVL-H3 at sites corresponding to nucleotides 593 and 2518 of RTVL-H2 (Ref.
Fig. 3. Possible splicing patterns in the synthesis of P25 as well as RTVL cDNA, cTP4. The genomic structure of calbindin is schematic. For P25, transcription starts presumably in the 5' LTR and proceeds toward the calbindin gene if the two domains are actually linked. The first RNA splice takes place between the 5' splice site ss of RTVL and the 3' splice site of exon 2 of calbindin. Exon 1 of calbindin does not have a 3' splice site. For cTP4, an RNA splice takes place between the 5' and 3' splice site within the RTVL domain. RTVL-H3 is not as full length as RTVL-H2 and contains an internal deletion, A. ORF, open reading frame; kb, kilobase.

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transcripts in the normal prostate, whereas a high message level is found in teratocarcinoma cells and in the chorion and amnion tissues of placenta (20). P25 is unique in that it results from a gene fusion between an endogenous retrovirus and a known cellular gene sequence via RNA splicing.

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CALBINDIN TRANSCRIPTS IN PROSTATE CELL LINE PC-3

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