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 (tRNA spacer) 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.

Construction of Subtraction Libraries. Isolation of RNA and construction of cDNA libraries were done following standard procedures (7). The cloning vector, pLOT731, is 3.7 kilobases long and contains sequences from the bacteriophage M13 that allow conversion of the plasmid DNA into a single-stranded form when pLOT731-transformed bacteria are coinfected with a helper phage, R408 (8). The plasmid also contains sequences that confer tetracycline resistance as well as part of the β-galactosidase gene. Insertion of cDNA into the β-gal gene inactivates it; this permits ready differentiation of recombinant from nonrecombinant clones. The cloning site has the following sequence: GAATTCCATATGGCTGGCAGCACAATTCCTGACG. Underlined are two BstXI restriction enzyme sites used in our cDNA construction. The host bacteria is JM107. Single-stranded plasmid DNA was prepared and biotinylated (9). To check that ssDNA has been biotinylated, a small sample was dot-blotted onto filter and treated with avidin:horseradish peroxidase (1:400) and 3,3′-diaminobenzidine tetrahydrochloride. Subtractive hybridization was carried out in 50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid HEPES, pH 7.6-0.2% sodium dodecyl sulfate 2 mM EDTA-0.5 nM NaCl. Biotinylated ssDNA was used in 10-fold excess of nonbiotinylated ssDNA. The hybridization solution was heated to 95°C for 2 min and incubated at 65°C overnight. Afterwards, the solution was extracted with phenol (10). The subtracted ssDNA was made double stranded by primed synthesis using the commercially available M13 sequencing 24-mer (AGCGGATACCAATTTACACAGG). 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 cloned inserts were either radiolabeled by nick translation or biotinylated for detection by chemiluminescence (Tropix Translight; Tropix, Bedford, MA; BRL Photogene Detection System, Bethesda, MD) (12).

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 ssDNA was biotinylated, and a 10-fold excess of the biotin-cDNA was hybridized to PC3 ssDNA. 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 ssDNA.

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. A. 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—L*—*G—L*—*-G—L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*-L*—*
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, Δ. ORF, open reading frame; kb, kilobase.

...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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