Molecular Cloning of an Alternative Human αE-Catenin cDNA

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The cytoplasmic protein alpha-catenin plays a crucial role in E-cadherin mediated cell-cell adhesion by binding E-cadherin to the cytoskeleton via beta- or gamma-catenin and actin. Functional loss of one of these interacting components leads to decreased cell-cell adhesion, and therefore to loss of epithelial integrity. Northern analysis revealed two distinct αE-catenin transcripts in different cell lines, whereas apparently only one protein is expressed. Because of the biological importance of this protein we sought to molecularly characterize the differences between the two observed transcripts. cDNA cloning and sequence analysis revealed the earlier described 3.4 kb αE-catenin transcript and an αE-catenin transcript of approximately 3.8 kb. This larger transcript contains a 321 bp extension in the 3'UTR sequence, which probably arises as a result of alternative polyadenylation. Considering the presence of AU-rich sequences in the extension, it may be involved in mRNA stability.
Two distinct αE-catenin transcripts with different relative expression levels have been observed in some cell lines, although, western analysis revealed a single 110 kD band, suggesting that only one α-catenin protein is expressed. In order to resolve the molecular basis for the complexity in the α-catenin mRNA expression pattern, we screened a human prostate cDNA library and identified two distinct human α-catenin cDNA transcripts. Here, we describe the molecular characterization of the differences between these two transcripts. Furthermore, we performed computer-assisted analysis of αE-catenin related expressed sequence tags (ESTs). This confirmed that the different transcripts arise due to alternative polyadenylation.

MATERIALS AND METHODS

Isolation of the human αE-catenin cDNA clones. A cDNA library was constructed from RNA isolated from the human cell line 267B1 (prostatic epithelial cell line derived from fetal prostate tissue (26)) using the ZAP-cDNA synthesis kit and ZAP-cDNA Gigapack 2 Gold cloning kit (Stratagene). The library was screened, using a 1.5 kb human α-catenin cDNA fragment as a probe (27), pBluescript SK(-) phagemids were obtained using helper phage R408 according to the in vivo excision protocol (Stratagene). A second cDNA library used, was constructed from human fetal kidney (Clontech).

cDNA sequence analysis. Conventional cloning methods were used to clone cDNA fragments into the plasmids pUC18 and pGEMZf(-) (27). The dideoxy termination method (28) was used for nucleotide sequence analysis. The gel readings were recorded and edited using IntelliGenetics computer software. Computer comparison studies were performed with sequences obtained from the EMBL and Genbank nucleotide sequence databases (29), using CAMMSA computer software.

EST alignment. αE-catenin specific EST (expressed sequence tags) sequences were selected from original ABI-sequence profiles from the Washington University-Merck EST project (genome.wustl.edu). These sequences were aligned and analyzed using Staden GAP4 software (30).

Northern and Southern blot analysis. Total RNA was isolated from the cell lines A431 (human cervix carcinoma cell line), LNCAP and PC3 (both human prostate cancer cell lines). 10 μg of total RNA was glyoxalated, separated on a 1% agarose gel, capillary transferred to a Hybond-N+ membrane (Amersham) and immobilized by uv-crosslinking.

Chromosomal DNA was isolated from the above mentioned cell lines. 10 μg of DNA was digested using endonuclease EcoRI, separated on a 1% agarose gel and capillary transferred to a Hybond-N+ membrane (Amersham).

The blots were hybridized according to Church and Gilbert (31) using either a human αE-catenin coding cDNA fragment of 1.5 kb, or a 350 bp 3'UTR specific fragment from clone 921 as a probe. Ribosomal RNA and γ-catenin were used as controls for the amounts of respectively total RNA and chromosomal DNA loaded. Probes were radioactive labeled with [α-32P]dATP by random prime labeling (32).

PCR analysis. PCR was performed in a reaction mixture of 50 μl containing 1X-PCR buffer (SphaeroQ), 0.2 mM of each dNTP (dATP, dCTP, dGTP, and dTTP), 0.5 pmoles of each primer, 1 mM MgCl2 (primer set P3-F4) or 2.5 mM MgCl2 (primer set P1-P2) and 1 unit Supertaq DNA polymerase (SphaeroQ). 2 ng plasmid DNA (positive control), 10 ng cDNA or 500 ng chromosomal DNA was used as a template. Using a Perkin Elmer thermocycler, the following program was performed: 1 min at 94°C, 1 min at 60°C, 1 min at 72°C, for 35 cycles followed by a final extension of 10 min at 72°C. The following αE-catenin specific primers were used (see also fig. 4A):

- sense primer P1: 5' GGG CAT CTC AGA AGA AGC AC -3'
- sense primer P2: 5' AAA TTC ACC ACC TGG ACT GG -3'
- sense primer P3: 5' GGG AAT TTT GCT CAA CTT CA -3'
- anti-sense primer P4: 5' GIT GTC AGG TTT TTC TGC TGC TG C -3'

10 μl of total PCR mixture was analyzed on a 2% agarose gel.

RESULTS

Isolation of Human α-Catenin cDNA Clones

Upon screening a human prostate cDNA library with the 1.5 kb human αE-catenin probe, we obtained 120 positive clones. Analysis of 20 clones suggested that 7 clones contain the full length human αE-catenin cDNA (data not shown). Sequence analysis of these 7 clones revealed that they contain the ATG start codon, followed by a 2718 bp open reading frame from the human αE-catenin cDNA as described by Furukawa et al. (19).

However, differences in 3'UTR sequences were observed (fig 1): clone 921 showed a 321 bp extension of the 3'UTR when compared to clone 1021, which is identical to the earlier described αE-catenin cDNA. Clone 1021 contains an AATAAA polyadenylation signal that starts at position 3413, clone 921 contains this sequence at the same position, but has a second polyadenylation signal at position 3731. Three out of seven sequenced clones contain the extension. Upon screening a human fetal kidney cDNA library, 6 out of 7 αE-catenin specific clones showed the originally described poly(A) addition site (19). The remaining clone was likely to be artificially primed.

EST Alignment

Database searches revealed 59 αE-catenin related ESTs available from the Washington University-Merck EST project. 32 sequences out of 59 ESTs contained clear polyadenylation tails, from which 27 start at 2 dominant positions i.e. for 13 ESTs at position 3434-3437 and for 14 ESTs at position 3755-3757 (fig 1). The position of the first predominant polyadenylation site is equal or nearby (+1 or +3 bp) to that of the previously described αE-catenin transcript (19), whereas the position of the second one is equal or nearby (+2 bp) to the one identified in clone 921. The poly(A) tail start sites of the remaining poly(A) tail containing αE-catenin specific ESTs are indicated in fig 1.

Northern Blot Analysis

In order to test the hypothesis that the extended transcript we identified is in fact the explanation for the earlier observed two distinct αE-catenin transcripts in different cell lines (33), we performed a northern blot analysis. Using an α-catenin cDNA probe, two transcripts of respectively 3.4 kb and 3.8 kb were ob-
FIG. 1. Nucleotide sequence analysis of the 3'UTR extension of clone 921. The sequence of the previously described αE-catenin cDNA (clone 1021) is in capitals (position 3401-3433). Clone 921 contains an extension of 321 bp that starts at position 3434. Poly-adenylation signals are underlined and the start-sites of the poly(A) tails are indicated by vertical lines.

As expected, no transcripts were detected in cell line PC3 (fig 2A, B) due to the homozygous deletion of the functional αE-catenin gene (22).

**Southern Blot Analysis**

To determine whether both αE-catenin forms are derived from one αE-catenin gene, a Southern blot analysis was performed. Using the coding cDNA probe it was shown that both the αE-catenin gene and the αE-catenin pseudogene are present in the cell lines A431 and LNCAP. Cell line PC3 contains the αE-catenin pseudogene only (fig 3A). Apparently the 90% similarity between the functional gene and the pseudogene is enough to detect the pseudogene in Southern analysis. From hybridization with the 3'UTR specific probe it became clear that the 3'UTR extension as identified in clone 921 is present in the αE-catenin gene, but absent in the pseudogene (no fragment detected in the lane containing PC3, fig 3B). The fact that the hybridizing fragment using the 3'UTR probe is of identical size (±9.5 kb) as one of the fragments using the coding probe, suggests that there is indeed only one gene responsible for the αE-catenin transcription. The origin of the larger fragment observed in the lane containing LNCAP chromosomal DNA (fig. 3B) is not clear.

**PCR Analysis**

To determine if the 3'UTR extension of clone 921 is a part of the last exon (exon 16) of the α-catenin gene, we performed a PCR analysis. The forward primer is located 5' to the first polyadenylation signal of the αE-catenin transcript (P3, fig 4A) and the reverse primer is located within the extended 3'UTR sequence of clone 921.
that besides the human αE-catenin cDNA as previously described (19) another human αE-catenin transcript was identified. This variant, represented in cDNA clone 921, showed an extension of 321 bp in the 3'UTR sequence and has a length of 3754 bp, when compared to the shorter, previously described transcript of 3433 bp. The newly identified transcript apparently results from using an alternative polyadenylation site. These data were confirmed upon aligning 59 sequences of αE-catenin EST clones. The total alignment revealed two predominant αE-catenin transcripts, with a length comparable to the transcripts we identified.

From the observation of two transcripts in the cell lines A431 and LNCAP (northern analysis) and the presence of only one αE-catenin gene (Southern analysis) it can be concluded that both transcripts are transcribed from the same gene. Furthermore, this provides the first evidence for alternative usage of polyadenylation signals in the expression of the αE-catenin gene. The finding that 6 out of 7 αE-catenin cDNA clones, derived from a fetal kidney library, showed the same poly(A) addition site, suggests some tissue specificity in the use of poly(A) addition signals. However, the 13 EST clones using the first poly(A) addition signal (position 3413) were derived from 8 different tissues, whereas the 14 EST clones using the second signal (position 3757) were derived from 11 tissues, of which 6 are shared with the first category. These preliminary data argues against high tissue-specific preference for a particular poly(A) addition signal.

**DISCUSSION**

Recent observations indicated that the αE-catenin gene is transcribed in at least two different mRNAs, even though only one protein seems to be expressed. This complex transcription of the α-catenin gene might be associated with functional regulation of the gene. In order to gain more insight into the molecular mechanism behind this phenomenon we isolated a number of human αE-catenin cDNA clones of different sizes from a prostate cDNA library. Sequence analysis showed that besides the human αE-catenin cDNA as previously described (19) another human αE-catenin transcript...
The αE-catenin isoforms identified before can be distinguished by a deletion (17) or an insertion (33) in the coding region of the α-catenin cDNA. The possible function of the larger 3'UTR which occurs in the non-coding region can therefore not be predicted from previous examples. Assuming that the relative expression levels of the two different transcripts are functionally relevant for the cells, it can be suggested that the 3'UTR extension has a regulatory function in mRNA stability. Studies by Zubiaga et al. (34) showed that different sequence motifs present in the 3'UTR of human genes can play a role in mRNA stability. For instance, the nonamer UUAUUUAUU is a AU-rich sequence motif that mediates mRNA degradation, whereas the length of the poly A tail might be involved in mRNA stability. Although the newly identified 3'UTR extension is an AU-rich sequence, no specific mRNA degradation accelerating or stabilizing elements can be distinguished.

In conclusion, we isolated an alternative human αE-catenin transcript which contains an additional 321 bp in the 3'UTR sequence. The extension probably arises due to alternative polyadenylation of the αE-catenin transcription unit. This additional sequence might play a role in the mRNA stability, however, further investigations will be necessary to confirm this hypothesis.

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REFERENCES


181