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Phylogenetic analysis, expression patterns, and transcriptional regulation of human CTEN gene

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Abstract

Cten is a focal adhesion molecule and a member of the tensin family. Its expression is highly enriched in the prostate and placenta, suggesting that cten gene might be closely associated with mammalian species. Recent studies have reported that cten expression is frequently up-regulated in a variety of cancers and its levels appear to correlate with tumorigenicity. Here, we have (1) analyzed cten sequences of various species to build a phylogenetic tree, (2) examined cten mRNA levels in human and mouse tissues to establish its expression profiles, and (3) determined the promoter region of human CTEN gene in cell lines and in a mouse model to understand its transcriptional regulation. Our analyses indicate that all currently known cten genes are present in mammals. The prostate and placenta are the two most cten abundant tissues in human and mouse, meanwhile brain and lung also express low levels of cten. Results from cell culture reporter assays demonstrate that a 327-bp fragment is the shortest functional promoter. All functional promoter constructs produce 40- to 160-fold increases in luciferase reporter activities in normal prostate cells, whereas lower activities (< 40-fold) are detected in non-prostatic cell lines. To evaluate CTEN promoter activity in mice and develop a new tissue specific Cre recombinase mouse model, we have established pCTEN-Cre:R26R mice by crossing R26R β -galactosidase reporter mice with pCTEN-Cre transgenic mice, in which the 327-bp cten promoter drives the expression of Cre recombinase. X-gal analysis has shown strong β -galactosidase activities in the prostate, brain, and few other tissues in pCTEN-Cre:R26R mice. Altogether, we have identified the promoter region of human cten gene and provided a useful tool for investigating cell lineages and generating tissue-specific knockout or knockin mice.

Keywords

cten/Tns4; phylogenetic; promoter; Cre mouse

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1. Introduction

Tensin is a focal adhesion molecule that links integrin receptors to the actin cytoskeleton and acts as a regulator for signal transduction (Lo et al., 1994; Lo, 2004). There are four members in the tensin family: tensin1, tensin2, tensin3 and cten (C-terminal tensin-like). They all share the similar C-terminal region containing a Src homology 2 (SH2) domain and phosphotyrosine-binding (PTB) domain (Lo, 2004). Unlike other members, the smallest protein cten (aka Tns4) lacks the N-terminal common region, which contains the actin-binding activity, one of the two focal adhesion-targeting regions, and the phosphatase-like domain (Lo and Lo, 2002; Lo, 2004). This unique protein structure of cten makes it a potential natural inhibitor or regulator of tensin-mediated signaling by competing SH2 and/or PTB binding molecules with other tensins. One example is that up-regulated cten displaces tensin3 from the cytoplasmic tail of integrin β 1, disrupts the linkage between actin cytoskeleton and focal adhesions, and promotes breast cancer cell migration and invasion activities (Katz et al., 2007).

Our previous studies have shown that cten is highly expressed in normal prostate and placenta and is not detectable in other tissues by Northern blot assays (Lo and Lo, 2002). Nonetheless, further studies have indicated that cten is up-regulated in many cancerous tissues, such as lung, thymus, breast, stomach, pancreas, and colon cancer (Sasaki et al., 2003a; Sasaki et al., 2003b; Katz et al., 2007; Sakashita et al., 2008; Liao et al., 2009; Al-Ghamdi et al., 2011; Albasri et al., 2011a; Albasri et al., 2011b), suggesting that cten may play an oncogenic role in these tissues. As mentioned above, cten is overexpressed in breast cancer and contributes to cancer cell invasiveness (Katz et al., 2007). In addition, up-regulated cten promotes colon cancer cell colony formation, anchorage-independent growth, and invasiveness by interacting with β -catenin and/or regulating ILK activity (Liao et al., 2009; Albasri et al., 2011a). Furthermore, cten expression induces sensitivity to paclitaxel in prostate cancer cells (Li et al., 2010). These findings strongly suggest that cten expression levels may dictate the process of cell transformation and drug resistance. Therefore, analysis of cten promoter region/activity will offer a better understanding of the mechanism regulating cten expression and provide a platform for potential clinical application in the future.

In this report, we have analyzed and built a phylogenetic tree of cten genes; examined cten expression patterns in human and mouse normal tissues, as well as in human prostate diseases by quantitative-PCR (Q-PCR) assays; validated the transcription initiation site by 5' RACE and ChIP-seq data analysis; and determined the promoter activities of human cten gene by reporter assays in cell culture systems and in a Cre-Lox mouse model. Our findings have indicated that (1) CTEN appears to be a newly evolved gene that is highly expressed in the prostate and placenta; (2) it is frequently down-regulated in human prostate cancer and benign prostatic hyperplasia; and (3) human CTEN promoter displays a tissue- and cell type-specific activity.

2 Materials and methods

2.1. Sequence and phylogenetic analysis of cten genes

The human cten protein sequence AAN32666.1 was selected from Genbank and queried with protein-protein BLAST via the NCBI web server (Altschul et al., 1997); 22 protein orthologs possessing sufficient identity across the query sequence and with blast scores > 200 were identified; their corresponding nucleotide coding regions (CDS) were used as the basis of the multiple sequence alignment (MSA). The program MAFFT (Katoh and Toh, 2008) generated the MSA via the EBI web server using minor changes to the default parameterization. Since MAFFT does not optimize the alignment under a codon constraint,

further adjustments were made with Los Alamos National Laboratories codon aligner (<http://www.hiv.lanl.gov/content/sequence/CodonAlign/codonalign.html>); it was assured that the start codon of the Homo sapiens sequence and the majority of other sequences (16/23) were designated as the beginning of the open reading frame; parameters were adjusted until no stop codons were present in the protein translation of the final alignment for any of the 23 sequences. The program GBlocks (Talavera and Castresana, 2007) primarily removed large gap regions while accounting for codon structure; 2007 of the 2595 sites (77%) were preserved under a stricter parameterization, while allowing for some gaps in the alignment. jModelTest (Posada, 2008) guided evolutionary model selection for phylogenetic tree construction; the TPM2uf+G model was the final model chosen (out of 88 models) under the criterion of having the lowest Akaike score (Akaike, 1974); the program phyML (Guindon et al., 2010) built the phylogenetic tree, which estimated the model parameters via maximum likelihood. The tree was rooted with the tasmanian devil and grey short-tailed opossum, marsupials which are well documented to serve as a suitable outgroup in mammalian phylogenies at Tree of Life Web Project (<http://tolweb.org/Eutheria/15997/1997.01.01>). HyPhy (Pond et al., 2005) served as a MSA viewer and file conversion to PHYLIP format. The tree was furnished with 1000 pseudoreplicate bootstrap values at each node (Felsenstein, 1985) and was edited for aesthetics in FigTree (<http://tree.bio.ed.ac.uk/software/figtree/>).

2.2. Cten expression profiling in human and mouse tissues

Ready-to-use normalized cDNA preparations from various human and mouse tissues were purchased from Clontech (Mountain View, CA). One μ l of cDNA was used for quantitative fluorescent real-time PCR with SYBR Green PCR master mix (Applied Biosystems, Warrington, UK) in the ABI Prism 7700 sequence detector (Applied Biosystems). The mRNA expression levels of cten in human and mouse were normalized by GAPDH. The primers used in PCR analysis were: human cten forward, 5'-ggggacccagaggaccttgac-3', human cten reverse, 5'-gggggaagcagctggaaggtgg-3'; human GAPDH forward, 5'-tgaagtcggagtcacggatttggt-3', human GAPDH reverse, 5'-catgtggccatgaggtccaccac-3'; mouse cten forward, 5'-ctgtatcggatgcagctatgtgtt-3', mouse cten reverse, 5'-agccactggagtagactgcactgct-3'; mouse GAPDH forward, 5'-aacatcatccctgcattccac-3', mouse GAPDH reverse, 5'-ctgcttcaccaccttcttga-3'.

TissueScan Prostate Cancer cDNA Array (OriGene Technologies, Rockville, MD) were used to examine cten mRNA expression profile. Relative quantification of cten expression was measured by normalization against β -actin using the ΔC_T method. Fold changes between the average of normal tissues (control) and diseased samples were quantified as $2^{-(\Delta C_T \text{ sample} - \Delta C_T \text{ control})}$.

Human placenta, prostate, skeletal muscle, and lung protein lysates were purchased from BioChain Institute (Newark, CA). Protein lysates (150 μ g) were loaded for immunoblotting analysis using cten and GAPDH antibodies.

GSE29079 containing large-scale gene expression profiles of 48 normal and 47 prostate tumor samples using Affymetrix GeneChip Exon 1.0 ST microarrays (Brase et al., 2011) was downloaded from the NCBI Gene Expression Omnibus. Cten expression profiles were analyzed using Partek Genomics Suite (Partek software, version 6.6 Beta). Dot plots were generated using the same software.

2.3. Cell culture and transfections

HEK293, A549, NIH3T3, SW1353 and SW480 cell lines purchased from American Type Culture Collection (ATCC) were cultured in DMEM supplemented with antibiotics and 10%

fetal bovine serum. RWPE-1 from ATCC and MLC-SV40, a gift from Dr. John Rhim (Uniformed Services University), were cultured in keratinocyte serum free medium (Invitrogen, Carlsbad, CA). Transfections in RWPE-1 and MLC-SV40 cell lines were performed using TransIT-prostate transfection kit (Mirus, Madison, WI) as described in manufacturer's instruction. Transfections in other cell lines were carried out using Lipofectamine 2000 (Invitrogen) according to the user's manual.

2.4. 5'-Rapid amplification of cDNA ends (5'RACE)

First strand cDNA was synthesized from MLC-SV40 cell total RNA using MMLV Reverse Transcriptase and CTEN primer 1 complementary to nucleotides 122-144 (ttcttactacaccaggaaggc) downstream from the putative ATG start site. After RNA template was removed by RNase H and RNase T1, a homopolymeric tail was added to the 3'-end of the cDNA using terminal deoxynucleotidyl transferase and dCTP. The tailed cDNA was amplified by PCR using the anchor primer and a nested primer: ccagcaccagccccagcctg (complementary to nucleotides 91-111 downstream to CTEN ATG site). The PCR products were cloned into pCR II TOPO vectors (Invitrogen) for DNA sequencing.

2.5. Plasmid construction and Luciferase assay for promoter activities

The 5' flanking region of human CTEN gene was subcloned into a promoterless, enhancerless pGL3-Basic luciferase reporter vector (Promega, Madison, WI). The various length of reporter constructs (1.44 µg) were transfected into indicated cell lines together with pRL-TK vector (0.16 µg) (Promega), which was to determine the transfection efficiency. Luciferase activities were measured by Dual-Luciferase reporter assay system (Promega) according to the technical manual. Briefly, cells were seeded one day before transfection and harvested 24 or 48 hours after transfection. Triplicate experiments were performed three times and the individual relative light units (RLU) from firefly luciferase were normalized to that from *Renilla* luciferase. The final value was expressed as the mean relative fold change (fold ± SD) compared with RLU from pGL3-Basic for each fragment.

2.6. Generation of pCTEN-Cre:R26R transgenic mice

To construct the cassette for the production of pCTEN-Cre transgenic mice, *SacII/EcoRI*-digested 327 bp fragment of human *cten* promoter was subcloned into the *SacII/EcoRI*-digested vector pOG231 (Addgene, Plasmid 17736 O'Gorman et al., 1991 carrying the Cre recombinase coding sequence fused with a nuclear localization signal. The final transgenic vector contains a 327-bp human *cten* promoter, synthetic intron sequence, Cre recombinase cDNA with nuclear localization signal, and an SV40 polyadenylation signal. This transgenic vector was purified using resin column (Qiagen, Valencia, CA), linearized, and sent to the murine targeted genomics laboratory (MTGL, UC Davis, CA) for pronuclear microinjection into inbred B6CBAF1 zygotes, which were then implanted into oviducts of pseudopregnant B6CBAF1 mice. R26R (*Rosa26Stop^{lox/lox} LacZ* reporter) mice purchased from Jackson Laboratories (Bar Harbor, ME) were cross-bred with pCTEN-Cre mice to generate the double transgenic pCTEN-Cre:R26R mice. All offspring were genotyped by PCR assay using specific primers for Cre transgene (forward primer F: 5'-cgtgcagggcccgaggaggaggag-3'; reverse primer R: 5'-ggatccgccgataaccagtgaacagc-3') and R26R allele (primers set: 5'-gcgaagagttgtctcaacc-3'; 5'-ggagcgggagaaatggatg-3'; 5'-aaagtcgctctgagttgttat-3').

2.7. X-gal staining

The major organs were dissected from pCTEN-Cre:R26R male or female mice and fixed in 0.2% glutaraldehyde for 4-6 hours. After washed in PBS, tissues were frozen and embedded in Tissue-Tek OCT compound (Sakura Finetek, Torrance, CA) using chilled isopentane and

stored at -80°C for further section. Seven- μ m sections were prepared for X-gal staining at pH 7.3 and histologic analysis as described previously (Chen and Lo, 2005). Tissues from age-matched R26R mice were used as the negative control for LacZ expression.

3. Results

3.1 Phylogenetic analysis of cten genes

By searching available databases at NCBI, we have found cten sequences from 23 different species, which had high protein homology with human CTEN sequence. All sequences are from mammalian species, suggesting that cten might be a mammalian specific gene. Analysis of these cten sequences allowed us to build a phylogenetic tree (figure 1), which agreed in large part with established taxonomic relationships.

3.2. Cten mRNA expression patterns in humans and mice

By Northern blot analysis, we previously found that cten was highly expressed in normal prostate and placenta (Lo and Lo, 2002). Since the sensitivity of Northern blot assay is limited, Q-PCR analyses have been performed to determine cten mRNA levels in human and mouse tissues. In agreement with previous findings, human placenta and prostate are two top cten-expressing tissues (figure 2A). In addition, lower cten levels are detected in human skeletal muscle, brain, kidney, lung, and thymus. In mouse tissues, cten is highly expressed in prostate and placenta as well (figure 2A). Other tissues including thymus, brain, testis, heart, and kidney also show weaker cten expression. Overall, the expression patterns of cten in humans and mice are very similar but not identical. To establish the relationship between cten mRNA and protein levels, we have analyzed cten protein expressions in human placenta, prostate, skeletal muscle and lung tissues (figure 2B). In agreement with mRNA levels, high and low levels of cten protein were detected in placenta and prostate, respectively. No signal was detected in muscle and lung samples.

The abundant cten expression in the prostate glands prompted us to investigate its expression in human prostate diseases. The Q-PCR studies using a total of 48 normal prostate, benign prostatic hyperplasia (BPH), and prostate cancer samples showed that cten was down-regulated (>4 folds) in 45% (14/31) of cancer and 55% of BPH (6/11) samples (figure 3A). By analysis of a publically available GSE29079 array data (Brase et al., 2011) containing 48 normal and 47 prostate tumor tissue samples (figure 3B), further confirmed that cten was significantly down-regulated in prostate cancers.

3.3. Identification of the potential transcription initiation site of human CTEN gene

The transcription initiation site of CTEN gene was determined by 5' RACE using total RNA isolated from human prostate cells MLC-SV40 (an immortalized, non-transformed prostate epithelial line). The 5' RACE products were cloned into pCR II TOPO vectors. Eight out of ten colonies picked from the plate contained inserts with similar sizes (270bp). Three clones were sequenced and all started at the same 5' end sequence, suggesting that this is the transcription initiation site. This 5' RACE approach allowed us to identify additional 39 bp upstream of our previously reported sequence (AF417488)(Lo and Lo, 2002).

Transcription initiation by RNA polymerase II (Pol2) involves binding of TBP (TATA binding protein) and assembly of TBP-associated factors (TAFs) at the initiation site. Recent genome-wide studies indicate that Pol2 accumulates at most promoters of protein-coding genes (Guenther et al 2007). By inspecting ChIP-Seq data at ENCODE (Encyclopedia of DNA Elements, <http://genome.ucsc.edu/ENCODE/aboutScaleup.html>), we found that the most prominent Pol-II occupancy, based on the ChIP-seq signals, at the human CTEN/TNS4 gene locus is around the first exon (figure 4), which is 69 bp and shares the same sequence

identified by our 5' RACE. Importantly, TBP and TAF1 also occupy the same short exon region. Transcriptional initiation by Pol-II is also linked to phosphorylation of Ser-5 at its CTD (Carboxy-Terminal Domain). Interestingly, occupancy by Pol-II with phosphorylated CTD at Ser-5, which was detected by the Pol2-4H8 antibody, is also located at the first exon. Together, these unbiased genome-wide studies strongly support our results of 5' RACE that this exon 1 is a transcription initiation site of CTEN.

3.4. Analysis of human cten promoter

To understand the mechanism of cten's high expression in prostate cells, we have analyzed the potential promoter activities of approximate 2 kb genomic DNA (-1976 to +37 bp) upstream of the first exon of human CTEN gene and shorter sequences derived from this region (figure 5). These DNA fragments were subcloned into the pGL3-Basic vector and were designated as pGL3-1976+37, pGL3-1010+37, pGL3-775+37, pGL3-480+37, pGL3-290+37, and pGL3-1010-130 (figure 5). The potential promoter activities were tested in two non-malignant prostate epithelial cell lines, MLC-SV40 and RWPE-1 (figure 5). All constructs except one were highly active (40-160 folds) in these two prostate epithelial cell lines. Among them, pGL3-775+37 exhibited the highest promoter activity and pGL3-290+37 was the shortest fragment that showed a strong activity. The construct pGL3-1010-130 had no luciferase activity indicating that the region from -130 to +37 was essential for the promoter activity, supporting our ChIP-seq finding (figure 4). In addition, these promoter constructs also showed mild activities (<40 folds) in SW1353 (human chondrosarcoma), HEK293 (human embryonic kidney epithelial cells), and SW480 (human colorectal adenocarcinoma), as well as weak activities (<20 folds) in A549 (human lung carcinoma) and NIH3T3 (mouse fibroblasts) cell lines. These results suggest that cten promoter is more active in prostate cells than other cell types.

Interestingly, there are two potential PSE binding sites (GGA[A/T]) within -290+37. Since PSE as well as cten are preferentially expressed in normal but not cancerous prostates (Nozawa et al., 2000), we tested whether these two PSE sites are functional and regulate the promoter activity in prostate cells. However, the PSE double mutations (-150 PSE: caggaaact to cagTGact, -94 PSE: cccttcc to ccTGtcc) did not compromise the luciferase activity of pGL3-290+37(PSE1/2), indicating that these two potential PSE sites do not contribute to CTEN promoter activity.

3.5 Determination of human cten promoter activity in pCTEN-Cre:R26R reporter mice

To determine whether the minimal human cten promoter region is functional in an animal, and whether it shows species and tissue specificities, we generated a transgenic mouse line pCTEN-Cre, in which the expression of Cre recombinase was driven by the shortest 327-bp human cten promoter (from -290 to +37 bp) region. Male pCTEN-Cre mice were crossed with female R26R reporter mice (Soriano, 1999), which carried an inactive LacZ reporter gene in the absence of Cre. In the presence of Cre, the loxed sequence region 5' upstream of LacZ reporter gene will be removed allowing the expression of LacZ gene. All pCTEN-Cre:R26R litters were born in healthy condition. No histological abnormality was detected in these transgenic mice during 24 months observation (data not shown).

X-gal staining was performed to detect β -galactosidase activities in various tissues. Overall, strong β -galactosidase activities were consistently observed in brain and prostate (figure 6). In the prostate, β -galactosidase activity was present in the anterior, dorsolateral, and ventral lobes of the prostate glands (figure 6A-C). In general, X-gal staining was strongest in the anterior lobes, moderate in the ventral lobes, and weakest in the dorsolateral lobes. Although the staining was consistently detected in both basal and luminal epithelial cells of these lobes, not all epithelial cells showed the X-gal staining. The X-gal positive cells were often

clustered. In addition, some surrounding fibromuscular cells also showed X-gal staining (figure 6A&B). No X-gal staining was detected in R26R prostate samples (figure 6D&E). In the brain of pCTEN-Cre:R26R, strong blue X-gal staining was shown in cerebellum, thalamus, hippocampus, choroid plexus, brainstem and cerebrum. In cerebellum, cells with strong β -galactosidase activity were detected in Purkinje cell layer but not in granular layer or other regions of the parenchyma (figure 6F). In dentate gyrus, strong β -galactosidase activity was present in some epithelial cells in the choroids plexus and some granular cells, whereas the moderate to weak activity was non-uniformly expressed in the cerebral parenchyma (figure 6G).

In addition to the brain and prostate, X-gal staining was occasionally found in islets of Langerhans in the pancreas (figure 6H), in the epithelium of bronchioles in the lung (figure 6I), and in some seminiferous tubes in testis (figure 6J). No consistent staining was found in kidney, small intestine, thymus, heart, tongue, liver, spleen, and mammary gland of pCTEN-Cre:R26R mice (figure 6 K-O, not shown). Taken together, these results have demonstrated that the human *cten* 327-bp promoter successfully drives the expression of Cre recombinase and activates β -galactosidase activities in a tissue specific manner in pCTEN-Cre:R26R mice.

4. Discussion

Cten has several unique features among tensin members. It only shares the conserved SH2 and PTB domains with other tensins and its molecular mass is about a half of other members. Cten expression pattern is much more restricted than other tensins. To date, all *cten* genes are from mammals, while other members for example *tensin1* is found in zebrafish, nematode, and even hydrozoan in addition to mammals. This might not be a coincidence, since *cten* is highly expressed in the placenta and prostate. The mammalian *cten* phylogenetic tree we built (figure 1) agrees in large part with established taxonomic relationships. Nonetheless, it does not perfectly match all species relationships perhaps because not enough intermediate linking species between clades were available. For example, the elephant is known to share a more recent common ancestor with horse, having diverged relatively recently than where it is currently lodged in the *cten* tree (Tree of Life Web Project, 1997, <http://tolweb.org/Eutheria/15997/1997.01.01>); yet the bootstrap score suggests it is firmly anchored at a more ancient divergence among the currently available *cten* sequences. If more sequences of the Proboscideans (i.e mammoths etc.) were available we might see a different topology. The majority of the bootstrap values for the tree in figure 1 inspire confidence of the topology, given this sampling of *cten* sequences. We further note that the *cten* gene appears to have undergone more divergent behavior in Rodentia more than any other clade as a whole. In contrast to most other leaves on the tree, the Human and Chimp sequences seems to have gone under little change since their most recent common ancestor. Looking ahead, this tree provides a foundation for studies in codon selection analysis.

By using cell culture reporter assays, we have identified a functional 2-kb human *cten* promoter and narrowed it down to a minimal 327-bp region that shows the strongest activities in prostate cell lines and weaker activities in other cell types. The promoter activity of the minimal 327-bp region is further demonstrated in the pCTEN-Cre:R26R mouse model. ChIP-Sequence data from ENCODE showing the occupancy of Pol2, TBP, and TAF1 also support the promoter function of this region. Nonetheless, our current studies do not exclude the possibility that alternative promoter usage for CTEN transcription may exist.

The analysis of pCTEN-Cre:R26R mice showed a tissue-specific X-gal staining pattern. The prostate, brain, pancreas, lung, and testis displayed strong X-gal staining, whereas kidney,

small intestine, liver, spleen, mammary gland did not show convincing staining. Since we used the Cre-Lox system in this mouse model, the X-gal expression pattern is not necessarily identical to that of cten promoter activity. It is known that Cre-mediated recombination is a unidirectional and cumulative process. In other word, the Cre-induced genomic DNA recombination will be present in all progeny of the recombined cell, even when the promoter is not driving Cre expression. The X-gal staining analysis reveals a mosaic pattern in pCTEN-Cre:R26R mice. For example, X-gal staining was detected in many but not all basal and luminal prostatic epithelial cells. These observations lead to following questions. Is this due to a technical issue by using the minimal promoter that is missing critical elements for a full activity? Or does this mosaic pattern represent a subtype of prostatic epithelial cells and reflect cell lineage relationships? To answer these questions, it will require intensive efforts and resources to generate and analyze reporter mice using longer cten promoter regions.

Our in vitro and in vivo promoter analyses provide the foundation for future studies on the cis- and trans- regulatory elements. This is especially important for potential clinical applications, since expressional profile analyses had shown that cten is frequently down-regulated in prostate cancer and BPH, while it is overexpressed in many other cancer types.

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Abbreviation list

Cten	C-terminal tensin-like)
SH2	Src homology 2
PTB	phosphotyrosine-binding
Q-PCR	quantitative-PCR
5'RAC	5'-Rapid amplification of cDNA ends
CDS	coding regions
MSA	multiple sequence alignment (MSA)
BPH	benign prostatic hyperplasia (BPH)
ENCODE	Encyclopedia of DNA Elements
Pol2	RNA polymerase II
TBP	TATA binding protein
TAFs	TBP-associated factors
CTD	Carboxy-Terminal Domain

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Highlights

1. A phylogenetic tree of mammalian cten genes was built.
2. Cten is highly expressed in the prostate and placenta.
3. Cten is down-regulated in human prostate diseases.
4. Human CTEN promoter region was identified.
5. A new tissue specific Cre transgenic mouse.

Figure 1. Phylogenetic analyses of mammalian cten genes

Cten sequences of 23 species were analyzed to generate the phylogenetic tree as described in Materials and Methods. The values represent bootstrap scores out of 1000 trials, indicating the credibility of each branch. The known cten gene loci for the species were indicated.

Figure 2. Cten mRNA expression patterns in human and mouse tissues

(A) The relative mRNA expression level of cten in human and mouse tissues. The results were expressed as ratios to the highest cten-expressed tissues in human and mouse. (B) Immunoblot analysis of protein lysates (150 ug) from human placenta, prostate, skeletal muscle, and lung by cten and GADPH antibodies.

Figure 3. Cten expression in human prostate samples

(A) Quantitative PCR analysis of cten transcripts in 48 normal, cancer, and BPH patient samples. Number labels in x-axis indicate different samples. (B) Dot plots of cten mRNA profiling of 48 normal (N) and 47 prostate tumor (T) sample data from GSE29079. ***P value < 0.0001.

Figure 4. CTEN transcription initiation region suggested by ChIP-sequence

A schematic representation of Pol2, TBP, and TAF1 occupancy around CTEN exon 1 region from ENCODE ChIP-Sequence data. The numbers next to the bars indicate the ChIP-sequence positions.

ttttactggtgaaatctaccaaacattttaggaagaaataggaccattctctacaacctctttcagaagatagaagcag
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 *
 Exon1

Figure 5. DNA sequence of the 5' regulatory region of the human CTEN

About 2-kb human genomic sequence 5' upstream of CTEN exon 1 (bold italic) is listed. Highlighted sequence within exon 1 shows the 39-bp identified by 5' RACE. The transcription start site is marked by an asterisk. Putative transcription factor binding sites are underlined.

Figure 6. Human CTEN promoter reporter activities in various cell lines

Schematic representation of the human CTEN promoter truncated constructs used in this study and their luciferase activities in various cell lines. Results were obtained from three independent experiments with triplicate and averaged.

Figure 7. X-gal staining of pCTEN-Cre:R26R or R26R mice

Tissues from 2 months old mice were used for the detection of β -galactosidase activities using X-gal staining (blue). X-gal staining was detected in anterior (A), ventral (B), dorsolateral (C) lobes of prostate in pCTEN-Cre:R26R mice. X-gal staining was found in basal (arrows) and luminal epithelial cells (white arrows) as well as fibromuscular cells (arrowheads). No staining was detected in R26R control anterior (D), ventral (E), and dorsolateral (not shown) prostate samples. X-gal staining was detected in cerebellum (F), dentate gyrus (G), pancreas (H), lung (I), and testis (J), but not in kidney (K), thymus (L), small intestine (M), heart (N), and tongue (O) of pCTEN-Cre:R26R mice. All X-gal stained sections were counterstained by Nuclear Fast Red. Scale bar, 100 μ m.