

SHORT COMMUNICATION

Identification and characterization of a new member of *serpin* family- HongrES1 in rat epididymis

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ABSTRACT

A full length cDNA named HongrES1 was isolated and cloned by screening rat epididymis cDNA library using a mouse EST as a probe and 5' RACE followed. It contained 1590bp nucleotides and its predicted protein had 415 amino acid residues including a serpin (serine protease inhibitor) conserved domain. Tissue distribution pattern showed it was specifically expressed in adult rat epididymis; moreover, in situ hybridization indicated this gene was expressed in a limited region of the cauda epididymis near vas deference. Such kind of expression pattern suggested that HongrES1 had potential function in male reproduction.

Key words: *HongrES1, serpin, epididymis, α_1 -antitrypsin, in situ hybridization.*

INTRODUCTION

Spermatozoa in testis lack forward motility and the ability to fertilize eggs, and the role of the epididymis is to provide an environment in which structurally and functionally immature spermatozoa leaving the testis can be converted into fully functional cells[1]. The epithelial cells form a luminal fluid environment by secreting proteins and fluid[2]. There is a complex serie of interactions between epididymal secretions and spermatozoa as sperms migrate along the epididymis[4]. About 150-200 proteins present in epididymal lumen[2]. Many of these proteins cannot be detected in other tissues or are present in the epididymis as specific isoforms. Thus a complete characterization of all the specific proteins secreted by the epididymis would yield important information for understanding the molecular events of sperm maturation. Many of epididymis

specific genes were found in monkey by our lab[3]. HongrES1 was one of the 'by-products' when we tried to isolate the homologue of the monkey epididymis specific gene ESc-615 by screening rat cDNA library. sequence analysis indicated it was a new member of serpin (serine protease inhibitor) family.

In mammals, serpin s are expressed in many tissues and they regulate the activities of a diverse array of serine and cysteine proteases, controlling complement activation, blood coagulation, fibrinolysis, inflammation, tumor cell metastasis, apoptosis, neurite extension, and many other physiological processes[5]. And it was also reported that several serpin s play important roles in male reproduction[6-8].

MATERIALS AND METHODS

Tissue samples

After the Sexually mature SD male rats were sacrificed, samples of epididymis, testis, kidney, liver, lung, heart, intestine, spleen, brain, vas deference, and prostate were obtained and immediately frozen in liquid nitrogen.

Probes and primers

P1(583): 5' GTGCCTTCTCACTTTCCTCA 3'

P2(144): 5' ATAAAGAGCAAACCTCCGGT 3'

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Received Jun-13-2002 Revised Oct-24-2002 Accepted Oct-27-2002

with Probe 1, and 25 positive plaques were obtained. One of the insert with the size of 1092bp was named as HongrES1. Blast search showed it was a new gene. Probe 2 could hybridized with a mRNA (about 1.6kb) in rat epididymis in Northern blot analysis (data not show), indicating this screening product was not a full length cDNA. A 620bp DNA fragment was got after 5' RACE. Altogether, the full-length of HongrES1 was 1590bp (Fig 1), and an ORF containing 415 amino acid residues was found by ORF Finder Program (<http://www.ncbi.nlm.nih.gov/gorf/gorf.html>) (Fig 1). There was a stop codon in the same reading frame upstream from ORF, and a signal peptide containing 45 amino acid residues was found by Signal Finder Program (<http://www.cbs.dtu.dk/services/SignalP>). A serpin signature from 377th amino acid residue to 387th amino acid residue

was found by the software at the site: <http://www.motif.genome.ad.jp/motif-bin/motif-markeq>. Blast search found HongrES1 had 33% identities with α 1-antitrypsin. This deduced amino acid sequence had conserved domain of SERPIN from 53rd amino acid residue to 404th amino acid residue by the program at

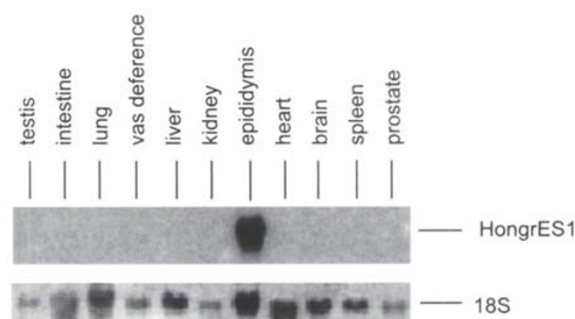


Fig 2. Expression of HongrES1 in different rat tissues

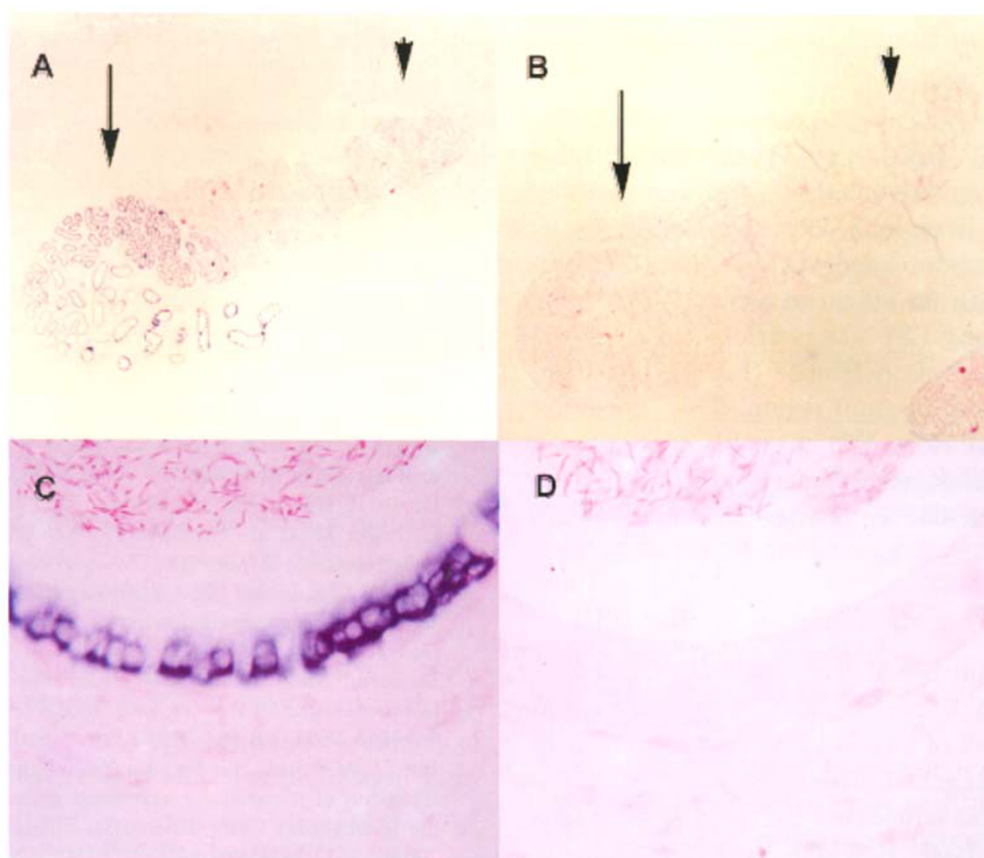


Fig 3. Localization of HongrES1 in rat epididymis by in situ hybridization (Blue regions were where HongrES1 was expressed) **A**, rat cauda epididymis (↑) and partial corpus (↑)(6.8×); **B**, rat caput epididymis(↑) and partial corpus (↑) (6.8×); **C**, epididymal duct in cauda hybridized with antisense of probe 3(40×); **D**, epididymis duct in cauda hybridized with sense of probe 3 (40×).

the site: <http://smart.embl-heidelberg.de/smart/show-motifs.pl>. Taking together, it shows a new member of serpin s family was found. Blast search showed HongrES1 had high homology (33%) with α 1-antitrypsin. The nucleotide sequence of HongrES1 has been submitted to the GenBank with accession number AY113703.

We predicted HongrES1 maybe not an inhibitory SERPIN, because the amino acids in the hinge region in RCL(reactive center loop) of HongrES1 were large polar residues (Lys, Asp, Glu, Se), but in inhibitory SERPIN these positions should be small residues such as Ala, Thr, Ser etc. Apart from the strange RCL sequence, HongrES1 had characteristics of α 1-antitrypsin: Thr and Leu were at P1 and P1' of RCL, and the protein contained most of the conserved residues[10]. But whether HongrES1 has inhibitory activity or not remains to be analyzed by experiments.

The expression of HongrES1 was epididymis specific in cauda region exclusively

Northern blot was used to determine the expression of HongrES1. Fig 2 showed that a strong signal was detected in epididymis, but there were not any signals in heart, liver, lung, kidney, intestine, testis, brain, spleen, prostate and vas deference. In situ hybridization with an antisense probe3 showed the expression of HongrES1 was restricted in a limited cauda region near vas deference (Fig 3), but there was not any signals at caput region. This expression pattern indicated HongrES1 probably played some roles in male reproduction. The research about HongrES1 will enrich our knowledge about the serpin family.

ACKNOWLEDGEMENTS

We express our heartfelt gratitude to Dr. James C. Whisstock in Department of Biochemistry and Molecular Biology of Monash University for his help in analysis of HongrES1 structure.

This work was supported by the Special Funds for Major State Basic Research Project ("973" No. G1999055901), State 863 High Technology R and D Project of China (No. 2001AA221211), Shanghai Development Foundation of Science and Technology (No. 01JC14046), Director foundation of Chinese Academy of Sciences, National Natural Science

Foundation (No. 30100064 & 39893320), Special funds of Biological Science and Technology from Chinese Academy of Science, NIH Forgarty International Center Grant 1-R03-TW01490-01A, CFDA No. 93.934, and CAS knowledge creative program (KSCX2-SW-201).

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