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European Journal of Cell Biology 84 (2005) 445–452
European Journal of Cell Biology
www.elsevier.de/ejcb

The cancer/testis antigen CAGE-1 is a component of the acrosome of spermatids and spermatozoa

Manfred Alsheimer*, Thomas Drewes, Wolfgang Schütz, Ricardo Benavente

Department of Cell and Developmental Biology, Biocenter, University of Würzburg, D-97074 Würzburg, Germany

Abstract

Cancer/testis antigens (CTAs) are characterized by their restricted expression pattern. In normal individuals their expression is largely restricted to the testis. In the case of cancer patients, CTA expression has also been frequently observed in the tumoral cells. CTAs are considered to be promising targets for immunotherapy. However, almost nothing is known about the properties defined by the vast majority of CTAs. Here, we have investigated the expression pattern and localization of the CTA CAGE-1 during mouse spermatogenesis. We show that protein CAGE-1 is 849 amino acids long. Analysis of the first spermatogenic wave of pubertal mice by RT-PCR and immunoblotting showed that CAGE-1 is predominantly expressed during postmeiotic stages. CAGE-1 localizes to the acrosomal matrix and acrosomal granule, as demonstrated by immunocytochemistry at the light and electron microscopic level. Taken together, our results allowed to define protein CAGE-1 as a novel component of the acrosome of mammalian spermatids and spermatozoa.

Keywords: Cancer/testis antigens; Spermatogenesis; Acrosome; CAGE-1; SCP1

Introduction

Cancer/testis antigens (CTAs) are characterized by their restricted expression pattern. In healthy organisms, CTAs are expressed in testis and occasionally in ovary and placenta. In the pathological situation, expression of CTAs has been also detected in many cancer tissues and cancer cell lines (for reviews see Old, 2001; Zendman et al., 2003; Scanlan et al., 2002). CTAs have the remarkable property to be immunogenic in cancer patients and due to the restricted expression pattern they are considered promising targets for cancer vaccines. Due to the potential use in cancer immunotherapy, the interest in CTAs has grown significantly in recent years. The number of identified CTAs is still increasing and more than 40 cancer/testis (CT) gene families involving over 80 individual genes or isoforms have been identified (for reviews see Old, 2001; Zendman et al., 2003; Scanlan et al., 2002).

Although CTAs have entered the phase of large-scale clinical trials (e.g. Davis et al., 2004), very little is still known about the proteins encoded by the CT genes. For the vast majority of the CT genes neither the expression pattern of the protein product, nor its localization and function in the germ line and tumoral tissues have been investigated. Some of the few examples of CTAs for which the expression and function in the germ line has been investigated are: (i) the synaptonemal complex protein SCP1, which plays a key role in chromosome synapsis during the first meiotic prophase (Meuwissen et al., 1992; Tüerci et al., 1998; Öllinger et al., 2005); (ii) the precursor of proacrosin-binding protein sp32 (or CTA

*Corresponding author. Fax: +49 931 888 4252.
E-mail address: alsheimer@biozentrum.uni-wuerzburg.de (M. Alsheimer).

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doi:10.1016/j.ejcb.2004.11.003

OY-TES-1) that is apparently involved in assembly of proacrosin in the acrosome matrix (Baba et al., 1994; Yamagata et al., 1998; Ono et al., 2001); (iii) C15/fertilin β, a member of the ADAM protein family, that might be important for the interaction between egg and sperm (Vidaeus et al., 1997; Primakoff and Myles, 2000); and (iv) the testis-specific small heat shock protein HSPB9 (de Witt et al., 2004).

Beside the interest in cancer immunology, CT genes and CTAs may be also relevant for cell and reproduction biologists. After all, we are dealing with a growing group of genes that in most cases code for unknown proteins of the germ line.

In search of putative structural proteins of mammalian germ line cells, we decided to characterize the protein product of CAGE-1 in mouse spermatogenesis. This CTA was recently identified by screening cDNA expression libraries with sera of patients with lung cancer (Park et al., 2003). The predicted sequence of the CAGE-1 protein contains long coiled coil domains as well as a putative nuclear localization signal (Park et al., 2003). It is worth mentioning that CAGE-1 is not related to another CTA called CAGE that was described previously by the same research group (Cho et al., 2002).

Materials and methods

Testis preparation

Testes from C57BL/6N mice were excised and transferred to a glass Petri dish containing ice-cold PBS (140 mM NaCl, 2.6 mM KCl, 6.4 mM Na_2HPO_4, 1.4 mM KH_2PO_4, pH 7.4). After removing the tunica albuginea seminiferous tubules were cut into small pieces and carefully resuspended with a syringe. In order to obtain a fraction of singularized cells enriched with spermatogenic stages, the suspension was filtered through a nylon filter (mesh size 25–30 μm) whereby remaining tubule pieces were removed. The cells were washed once with ice-cold PBS, counted, centrifuged for 10 min at 450*g* at 4 °C and immediately processed for either RNA isolation or protein analysis (see below).

Computational analysis and cDNA cloning

The human CAGE-1 cDNA sequence (GenBank™ accession number AF414185) was used as query for searching murine homologues in the NCBI databases by the Blast algorithm. One hit, a genomic contig (accession number NT_039579), was found to have high similarity to the query and seemed to contain the complete coding region. The computational predicted mRNA sequence (accession number XM_356655) showed a 5′ longer open reading frame, compared to the human sequence, that was verified by rescreening the databases for corresponding cDNA sequence and EST entries. For cloning full-length murine CAGE-1 cDNA, total RNA from testis of an adult mouse was isolated using TriFAST™ (PeqLab, Erlangen, Germany). Based on the predicted sequence a 5' oligonucleotide, corresponding to the region of the translation start (5′-GAAAGTATGTCAGAGTCGGAAACAA-TAAATGTC-3′), and a 3′ oligonucleotide, complementary to a sequence within the 3′ untranslated region including the stop codon (5′-ACGAATGAATATT-TAATCATGTATTACTGTCAGTTCA-3′), were selected and cDNA was amplified from 1 μg template RNA using the Superscript™ One-Step RT-PCR system (Invitrogen, Karlsruhe, Germany). The obtained cDNA was cloned into pCR®2.1-TOPO® (Invitrogen) and subsequently sequenced according to standard protocols. The deduced peptide sequence was computational analyzed using PSORT II prediction tool (http://psort.ims.u-tokyo.ac.jp/form2.html) and sequence motifs were scanned using the PPsearch tool (http://www.ebi.ac.uk/ppsearch).

Antibody production

To generate antibodies against murine CAGE-1 a 15-amino-acid peptide of the C-terminal region (RPRPRLDNHPKSLTL) was synthesized and two rabbits were immunized (SEQLAB, Göttingen, Germany) according to standard protocols. The sera were tested for their specificity either by immunofluorescence on testis cryosections or by immunoblotting. The specificity could be verified as the specific signals were blocked by adding the peptide used for immunization prior to incubation with the samples (not shown). For all applications we used affinity purified antibodies obtained from the polyclonal serum of one of the animals. For this purpose, a PCR product of murine CAGE-1 coding for aminoacids 820–849 was cloned into GST-fusion vector pGEX-5X-1 (Amersham Pharmacia, Braunschweig, Germany) and transformed into *Escherichia coli* XL1-Blue (Stratagene, La Jolla, CA). Bacterial expression of the GST-fusion protein was induced as described elsewhere, and the expressed protein was purified using glutathione-Sepharose® 4B (Amersham Pharmacia) according to the manufacturer's instructions. The fusion protein was coupled to CNBr-activated Sepharose™ 4B (Amersham Pharmacia) and the serum was affinity purified according to standard procedures. Antibodies against synaptonemal complex protein SCP1 were described elsewhere (Öllinger et al., 2005).