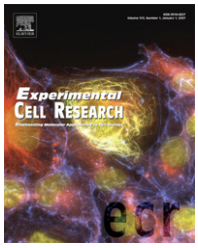
EXPERIMENTAL CELL RESEARCH 313 (2007) 1373–1392

available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/yexcr



Research Article

Coxsackie and adenovirus receptor (CAR) is a product of Sertoli and germ cells in rat testes which is localized at the Sertoli–Sertoli and Sertoli–germ cell interface

Claire Q.F. Wang[a], *Dolores D. Mruk*[a], *Will M. Lee*[b], *C. Yan Cheng*[a,*]

[a]*Center for Biomedical Research, The Population Council, 1230 York Avenue, New York, NY 10021, USA*
[b]*Department of Zoology, University of Hong Kong, Hong Kong, China*

ARTICLE INFORMATION

Article Chronology:
Received 30 August 2006
Revised version received
26 December 2006
Accepted 23 January 2007
Available online 3 February 2007

Keywords:
Testis
Spermatogenesis
Sertoli-germ cell interaction
Blood–testis barrier
Tight junction
Adherens junction
Ectoplasmic specialization

ABSTRACT

The coxsackie and adenovirus receptor (CAR), a putative cell–cell adhesion molecule, has attracted wide interest due to its importance in viral pathogenesis and in mediating adenoviral gene delivery. However, the distribution pattern and physiological function of CAR in the testis is still not clear. Here, we identified CAR in Sertoli cells and germ cells of rats. *In vivo* studies have shown that CAR resides at the blood–testis barrier as well as at the ectoplasmic specialization. The persistent expression of CAR in rat testes from neonatal period throughout adulthood implicates its role in spermatogenesis. Using primary Sertoli cell cultures, we observed a significant induction of CAR during the formation of Sertoli cell epithelium. Furthermore, CAR was seen to be concentrated at inter-Sertoli cell junctions, co-localizing with tight junction protein marker ZO-1 and adherens junction protein N-cadherin. CAR was also found to be associated with proteins of Src kinase family and its protein level declined after TNFα treatment in Sertoli cell cultures. Immunofluorescent staining of isolated germ cells has revealed the presence of CAR on spermatogonia, spermatocytes, round spermatids and elongate spermatids. Taken together, we propose that CAR functions as an adhesion molecule in maintaining the inter-Sertoli cell junctions at the basal compartment of the seminiferous epithelium. In addition, CAR may confer adhesion between Sertoli and germ cells at the Sertoli–germ cell interface. It is possible that the receptor utilized by viral pathogens to breakthrough the epithelial barrier was also employed by developing germ cells to migrate through the inter-Sertoli cell junctions.

Introduction

CAR is a 46-kDa transmembrane protein that enables viral attachment and entry into cells for coxsackie virus group B and adenovirus groups 2 and 5 [1]. The availability of CAR on cell surface is a determining factor of a cell's susceptibility to adenoviral vectors for gene delivery [2]. Therefore, extensive studies have been carried out to establish the expression profile of CAR in a variety of human tissues that are of interest to gene therapy, such as brain, heart and muscles [3–6].

* *Corresponding author*. Fax: +1 212 327 8733.
E-mail address: Y-Cheng@popcbr.rockefeller.edu (C.Y. Cheng).

0014-4827/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.yexcr.2007.01.017

As a structural component of tight junction and/or adherens junction, CAR is engaged in homotypical trans-interaction at regions of cell–cell contact, promoting cell adhesion and tissue genesis [6–8]. It has been found to associate with scaffolding proteins ZO-1 and β-catenin [8,9]. With two immunoglobulin-like domains in the extracellular region, a single transmembrane domain and a cytoplasmic tail [1,10], CAR joins JAMs and nectins to become a member of the immunoglobulin (Ig) superfamily. Integral transmembrane proteins of Ig superfamily are implicated in cell adhesion and migration. For example, JAM-C was described to promote neutrophil trans-endothelial migration [11], whereas nectin-like molecule-5 has been shown to enhance cell movement in NIH3T3 cells [12].

In adult rat testes, preleptotene spermatocytes traverse the blood–testis barrier from the basal to the adluminal compartment of the seminiferous epithelium for further development [13]. While they migrate progressively towards the lumen, spermatocytes differentiate into round and elongate spermatids until they detach from the epithelium at stage VIII of the epithelial cycle at spermiation [13]. This movement of germ cells involves rapid disassembly and reassembly of Sertoli–Sertoli and Sertoli–germ cell junctions [14]. Transmembrane proteins at the Sertoli–germ cell interface, for example, cadherins, nectins, integrins and JAMs, function as anchoring devices to maintain attachment between the two types of cells. More importantly, these proteins work in concert to facilitate the movement of germ cells [15]. To date, knockout studies of nectin-2, nectin-3 and JAM-C have yielded mice that were defective in spermatogenesis [16,17,18], illustrating the essential roles of these adhesion molecules in spermatogenesis.

Due to the structural similarity between CAR, JAMs and nectins, we aimed to investigate the presence of CAR in different cell types of the testis and its physiological significance to spermatogenesis. A recently published study has identified CAR at the acrosome region of mouse and human spermatozoa, as well as its interaction with JAM-C [19]. However, the presence of CAR at the Sertoli–Sertoli cell interface or tight junctions at the blood–testis barrier is not clear, nor do we know for certain about its expression in germ cells during their differentiation in the testis. In this report, we carried out *in vivo* and *in vitro* experiments to examine the cellular localization of CAR in Sertoli and developing germ cells, as well as its expression pattern during testicular maturation. In addition, we studied the interaction of CAR with peripheral regulatory proteins and the effects of cytokines treatment (e.g. TNFα) on its expression level in primary Sertoli cell cultures. These data will help elucidate the physiological role of CAR as a cell adhesion protein in the seminiferous epithelium during spermatogenesis.

Materials and methods

Animals

Male Sprague-Dawley rats were obtained from Charles River Laboratories (Kingston, NY). Rats were sacrificed by CO_2 asphyxiation. The use of animals for this study was approved by the Rockefeller University Animal Care and Use Committee with Protocol Numbers 00111, 03017 and 06018.

RT–PCR

Total RNA was extracted from tissues or cells by Trizol Reagent (Invitrogen). About 2 μg of total RNA was reverse transcribed into cDNAs using 0.3 μg of oligo$(dT)_{15}$ with a Moloney murine leukemia virus reverse transcription kit (Promega) in a final reaction volume of 25 μL. PCR reaction mixture was composed of 2–3 μL of RT product, with 0.4 μg of both the sense and anti-sense primers targeted to CAR (see Table 1). Co-amplification of rat ribosomal S16 gene was included to ensure the quality of RT product and the correct composition of each reaction mixture. The cycling parameters used in amplifying CAR are as follows: denaturation at 94 °C for 1 min, annealing at 58–59 °C for 1 min, and extension at 72 °C for 1.5 min, for a total of 26 cycles. After the reaction, 10 μL aliquots of PCR product were resolved by 5% T polyacrylamide gels using 0.5× TBE (45 mM Tris–borate, 1 mM EDTA, pH 8.0) as a running buffer.

Antibodies

Primary antibodies purchased from different vendors are listed in Table 2. Each antibody used in this study was shown to cross-react with its corresponding rat protein in our preliminary experiments. Bovine anti-rabbit IgG, bovine anti-goat IgG and goat anti-mouse IgG conjugated to horseradish peroxidase were purchased from Santa Cruz Biotechnology. The rabbit anti-CAR (H300) polyclonal antibody used in this study was raised against amino acid residues 1–300 mapping the N-terminus of CAR from human origin, which cross-reacted with the rat protein as indicated by the manufacturer. The two predominant isoforms of CAR differ only at the extreme C-terminus of the intracellular tail [10,20,21], therefore the anti-CAR (H-300) IgG detected both variants of this protein.

Primary testicular cell cultures

Sertoli cells

Sertoli cells were isolated from 20-day-old rats as previously described [22]. Freshly isolated cells were cultured at high cell

Table 1 – Primers for RT–PCR analysis of CAR and S16

Gene	Primer sequence	Orientation	Position	Length (bp)	Reference
CAR	5′-GGAAACTGCCTATCTACCCTGCAA-3′	Sense	173–196	531	GenBank Accession
	5′-CTGTAGGTCCCAGAATACTCAGAACT-3′	Anti-sense	678–703		Number: NM_053570
S-16	5′-TCCGCTGCAGTCCGTTCAAGTCTT-3′	Sense	15–38	385	[56]
	5′-GCCAAACTTCTTGGATTCGCAGCG-3′	Anti-sense	376–399		