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# Testicular cell adhesion molecule 1 (TCAM1) is not essential for fertility

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ABSTRACT

Testicular cell adhesion molecule 1 (*Tcam1*) is a testis-expressed gene that is evolutionarily conserved in most mammalian species. The putative location of TCAM1 on the cell surface makes it an attractive contraceptive target to study. We found that *Tcam1* transcription is enriched in the adult testis, and *in situ* hybridization revealed that *Tcam1* is expressed in pachytene to secondary spermatocytes. Immunofluorescence for TCAM1 protein showed strong expression along cell membranes of spermatocytes and weak localization to round spermatids. In light of this evidence, we hypothesized that TCAM1 interacts with an unknown receptor on the surface of Sertoli cells and that this interaction is important for germ cell-Sertoli cell interactions. However, *Tcam1* knockout mice that we generated are fertile, and testis weights and sperm counts were not significantly altered. Therefore, we conclude that TCAM1 is not essential for male fertility or germ cell function in *Mus musculus*.



## 1. Introduction

It has been estimated that there are over 2000 testis-expressed genes that may be essential to male fertility (Schultz et al., 2003). The discovery of the function and relevance of these testis-enriched genes has been a focus of our laboratory for a number of years (Yan et al., 2002, 2004; Greenbaum et al., 2006; Lin et al., 2007; Roy et al., 2007). To study the etiology of genetic causes of infertility and to identify potential contraceptive targets, we have used both *in silico* approaches and traditional gene expression analyses to identify evolutionarily conserved genes with gonadal-specific expression and interesting functional domains (Lin and Matzuk, 2005). One of the testis-specific genes that we identified in this manner is testicular cell adhesion molecule 1 (*Tcam1*).

In the literature, *Tcam1* has been described in detail in a single report (Sakatani et al., 2000). Sakatani et al. (2000) described mouse TCAM1 as a protein composed of a signal peptide sequence, five immunoglobulin (Ig) domains, and a transmembrane plus cytoplasmic domain. Northern blot analysis showed that *Tcam1* mRNA was present in the testis at postnatal day 17 (P17) and in adults but not at P12 leading the authors to conclude that *Tcam1* was predominantly expressed in pachytene spermatocytes and round spermatids (Sakatani et al., 2000); however, this was never confirmed by *in situ* hybridization. The authors also showed the close homology of the Ig domains in TCAM1 to those in ICAM1 and ICAM2 (Sakatani et al., 2000).

ICAM1 and ICAM2 are intercellular adhesion molecules that are important mediators of transendothelial migration of leukocytes in response to inflammatory stimuli (van Buul et al., 2007). Overexpression of ICAM1 *in vitro* promotes transmigration of neutrophils suggesting that the expression of this receptor is sufficient to drive leukocyte transendothelial migration (Sans et al., 2001), and conversely, blocking ICAM1 decreases transendothelial migration in a variety of models (van Buul et al., 2007). The binding of leukocyte-expressed integrins to endothelial-expressed ICAMs induces a variety of intracellular signaling events important for transendothelial migration (van Buul et al., 2007).

In the testis, junctional components of germ cells and Sertoli cells form dynamic interfaces that are constantly remodeled, replacing one component for another as the germ cells mature (Mruk and Cheng, 2004). Some important protein–protein interactions that form these junctions, such as nectin-afadin and integrins-ADAMs, are similar to protein–protein interactions that are important for contact between immune cells and their targets cells (Seals and Courtneidge, 2003; Mruk and Cheng, 2004; Fuchs and Colonna, 2006). Since ICAMs represent a similar class of molecules that are important for immune cell binding to target cells, we hypothesized that TCAM1 acts in a homologous way to ICAMs in transendothelial migration to aid germ cell migration

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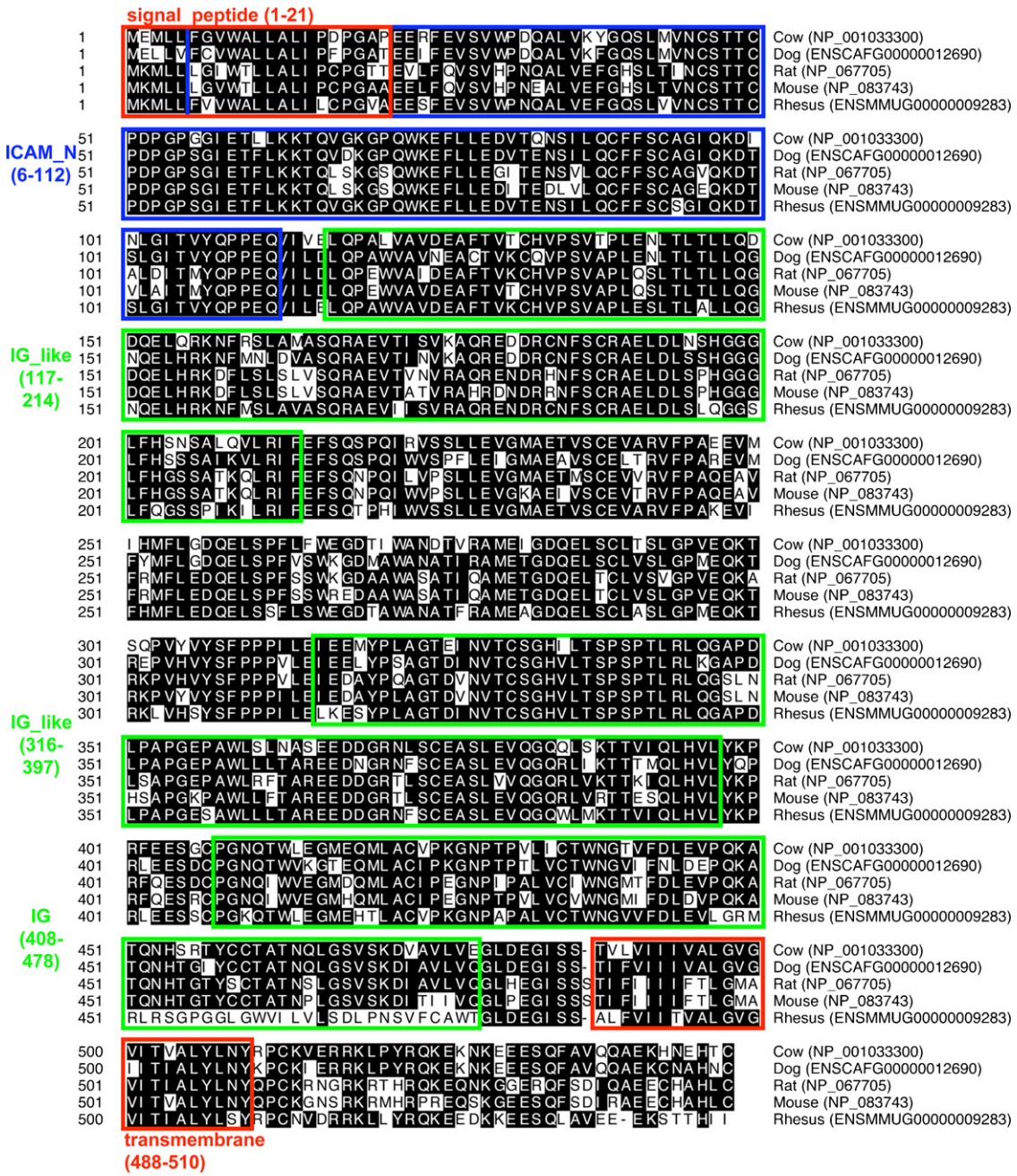


**Fig. 1.** Alignment and protein structure of TCAM1. Amino acids matching the consensus sequence are highlighted in black. Protein domain structure is annotated based on the mouse TCAM1 sequence (NP_083743.2) using SMART (http://smart.embl.de, Schultz et al., 1998).

and maturation within the seminiferous tubule. In this report, we sought to confirm and expand the previous literature on *Tcam1* using additional techniques including *in situ* hybridization, immunofluorescence, and knockout mouse technology.

## 2. Materials and methods

### 2.1. Alignments

Predicted amino acid sequences of TCAM1 for cow (NP_001033300.1), rat (NP_067705.1), and mouse (NP_083743.2) based on mRNA sequences were obtained from Entrez Gene (http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene). Predicted amino acid sequences of TCAM1 for dog (ENSCAFG00000012690) and rhesus monkey (ENSMMUG00000009283) were based on hypothetical mRNA sequences obtained from Ensembl (http://www.ensembl.org). Protein sequences were aligned using the multiple alignment algorithm, CLUSTAL V (MegAlign, DNAStar, Higgins and Sharp, 1989).

### 2.2. RNA isolation and semi-quantitative RT-PCR

RNA was extracted using TRIzol reagent according to the manufacturer's protocol (Invitrogen) and was converted to cDNA using SuperScriptIII primed by random hexamers (Invitrogen). *Tcam1* expression was examined at the exon 7–8 junction (Fwd, 5′-CTCCGTCAGCAAAGACATCA-3′; Rev, 5′-CATGCCAGGCTATTTCTGGT-3′) for multi-tissue semi-quantitative RT-PCR and the exon 2–3 junction (Fwd, 5′-AATGCTTCTGTTGGGTGTCTG-3′; Rev, 5′-GAGGGTAAGGGTGAGGCTCT-3′) to ensure efficacy of targeting. *Hprt1* (Fwd, 5′-CCTGGTTAAGCAGTACAGCC-3′; Rev, 5′-TACTAGGCAGATGGCCACAG-3′) and *Gapdh* (Fwd, 5′-AACTTTGGCATTGTGGAAGG-3′; Rev, 5′-ACACATTGGGGGTAGGAACA-3′) were utilized as endogenous loading controls. All product sizes and sequences were validated and reflected the expected results.

### 2.3. Histology, in situ hybridization, and immunofluorescence

Tissues were fixed in Bouin's fixative or 4% paraformaldehyde prior to paraffin embedding. Tissue embedding, sectioning, and staining for periodic acid Schiff