Original Article

Mouse CD52 is Predominantly Expressed in the Cauda Epididymis, Regulated by Androgen and Lumicrine Factors

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Abstract

Background: Sperm maturation takes place through contact between sperm and proteins produced in the epididymal lumen. CD52 had been characterised in the sperm; however, the expression and its regulation in the epididymis are mostly unknown. **Aim:** This study aimed to analyse the expression and regulation of CD52 in the mouse epididymis. **Setting and Design:** Experimental design was used in this study. **Materials and Methods:** Epididymis tissues from mice strain Deutch Democratic Yokohama were used as sources of total RNA. Bioinformatic tool was used to predict signal peptides. Quantitative real-time reverse transcription–polymerase chain reaction was used to analyse tissue distribution, androgen, testicular factors dependency and postnatal development. **Statistical Analysis:** One-way analysis of variance was used to analyse differences between treatment and control untreated group. $P < 0.05$ was determined as a significant difference. **Results:** CD52 amino acid sequence contains a signal peptide, indicating it is a secretory protein. CD52 exhibited region-specific expression in the epididymis, with the highest level being in the cauda. CD52 expression was regulated by androgen indicated by a significant downregulation at day 1 and day 3 following a castration ($P < 0.05$). Dependency on androgen was confirmed by injection of exogenous testosterone which prevented downregulation by 50%. Moreover, lumicrine factors also influenced CD52 expression indicated by ligation of efferent duct which also reduced expression at day 1 to day 5 following the ligation ($P < 0.05$). CD52 expression was developmentally regulated. This was shown by increase in the level of expression starting at day 15 postnatally. **Conclusion:** CD52 shows characteristics of genes involved in sperm maturation in the epididymis.

Keywords: *Androgen; CD52; Epididymis; Lumicrine, Sperm maturation*

Introduction

Spermatozoa leaving the testis are immotile and do not have the capacity to fertilize the oocyte. Spermatozoa must transit in the epididymis to undergo maturation process leading to molecular changes on sperm proteins. This process changes sperm to be functional because they have motility and Fertilizing capacity.[1,2] Previous studies showed that sperm maturation process takes place by contact between sperm and proteins produced by epididymal epithelium.[3] These proteins are a product of region specific expression of epididymal genes that create a microenvironment in which sperm cells come into contact with the proteins in a specific sequence.[4,5] Moreover, expression of epididymal genes involved in the sperm maturation is regulated by endocrine and lumicrine factors.[3] Therefore, identifying epididymal genes regulated by androgen is important in searching for those having

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a role in sperm maturation process. Examples of epididymal genes that are regulated by androgen are HongrES1 in cauda[6] and *Spag11a* in caput.[7]

Besides androgen, some reports suggested that lumicrine or testicular factors such as fibroblast growth factor (FGF) and androgen-binding protein (ABP) also regulate gene expression in the epididymis. Those regulated by testicular factors include *Cystatin 8* and *Lipocalin 8*.[4]

One of the genes putatively involved in the sperm maturation process is CD52. CD52 was first identified in the lymphocytes and it was found to be involved in signal transduction.[8] Expression of CD52 has been reported in mammals such as in dogs, rats, mice, monkeys and human. Genes for mouse CD52 or *B7* antigen is homologous with CD52 or HE5 in human.[9]

Expression regulation of CD52 in the epididymis has not been fully elucidated. Regulation of these genes is important to predict its role and to understand the mechanism of sperm maturation at a molecular level. Some literature showed that CD52 is involved in immunology reproduction.[5] On the other hand, Yamaguchi experiments showed that CD52 was not required for fertilisation in a mouse.[10] Therefore, this gene needs re-evaluation related to its putative function.

Based on the previous studies, it is important to understand the role of CD52 in the epididymis. This study was aimed to analyse CD52 sequence for secretory signal, tissue specificity and its expression regulation such as androgen dependency, lumicrine factors and postnatal development.

Materials and Methods

Animal model

Eight-week old male mice strain Deutch Democratic Yokohama were used throughout this study. The mice were fed food and water *ad libitum* in a place with 12 h of light, 12 h of darkness and temperature at 27°C. This study was approved by the Ethics Committee for Medical Research, No. 761/UN2.F1/ETIK/2014. All institutional and national guidelines for the care and use of laboratory animals were followed.

Analysis bioinformatics

The NCBI GenBank database was used to obtain full sequence of CD52. Primers were designed using Primer3 program (http://bioinfo.ut.ee/primer3-0.4.0/). The presence of signal peptide and location of cleavage were predicted using the SignalP 4.0 Server (http://www.cbs.dtu.dk/services/SignalP/).

Tissue distribution analysis

Mice were sacrificed and dissected through the abdomen to get various tissues, namely the testicle, four epididymal regions: the initial segment, caput, corpus and cauda, vas deferens, kidney, spleen, brain, muscle, heart, liver and intestine. Tissues were isolated and stored in RNA later until RNA isolation.

Mice castration

To analyse androgen regulation on CD52 expression, 24 mice were randomly distributed into six groups, with four mice for each group. The groups were control (not castrated), 6 h after castration and day 1 and day 3 after castration with testosterone injection. The castration was carried out by making an incision at the mouse abdomen. The mouse was under anaesthesia of 2.5% 2,2,2-Tribromoethanol (Sigma-Aldrich, St Louis, MO, USA). Testes were pulled out from the abdominal opening, blood vessels around the testis were pinched using hot tweezers and both testes were cut using sterile scissors, whereas the epididymis and vas deferens were put back inside the abdominal cavity. The incision was then stitched using sterile thread. Post operation, mice were put on top of a warm plate until they woke up. For the groups receiving androgen replacement therapy, testosterone (Sigma-Aldrich, St Louis, MO, USA) was injected intraperitoneally at a dose of 12 mg/kg/day starting at day 0, immediately after the operation. At the end of each group treatment, the mice were sacrificed and the epididymides were collected and total RNA was extracted.

Efferent duct ligation

Regulation by factors originating from testis and lumen of the efferent duct was investigated by efferent duct ligation (EDL). Fifteen mice were randomly distributed into five groups, with three mice for each group, namely the untreated control (unligated), 6 h and 1, 3 and 5 days with EDL. Ligation was done by tying the efferent duct using a synthetic polypropylene suture 6-0 (Prolene, Somerville, NJ, USA).

Postnatal expression analysis

This experiment was used to analyse CD52 expression throughout developing ages. Epididymis tissues were collected from mice with different postnatal ages of 5-, 10-, 15-, 22-, 30-, 40- and 60-day old mice. Mice were sacrificed and total RNA from the epididymis was isolated.

RNA extraction and quantitative real-time reverse transcription–polymerase chain reaction

Total RNA from all the tissues used in this study was extracted using a High Pure RNA Tissue kit (Roche, Penzberg, Upper Bavaria, Germany). Total RNA (10 ng) was used in real-time reverse transcriptase–polymerase

chain reaction (RT-PCR) analysis for expression distribution, androgen dependency, testicular factor regulation and postnatal development. Real-time reverse transcriptase PCR was performed using a universal One Step qRT-PCR Kit (KAPA Biosystems, Wilmington, MA, USA). Samples were run in triplicate together with internal standard control mouse beta-actin (Actb). Primers used in this study were CD52 forward (CTGCTACAGAGCCCAGGAAG) and CD52 reverse (GGTGGAGGTGCTGTTTTTGT), both primers have an annealing temperature of 58°C. For the internal standard, mouse beta-actin was used to normalise CD52 expression. Beta-actin primers used in this study were Act_F (CTAAGGCCAACCGTGAAAAG) and Act_R (CCATCACAATGCCTGTGGTA).

Statistical analyses

Data was analysed using Shapiro–Wilk test for normality. All statistical analyses were performed using one-way analysis of variance followed by Fisher's least significant difference (LSD) *post hoc* comparison test to determine which values differed significantly. The data was considered statistically significant at $P < 0.05$.

Results

CD52 has a signal peptide

SignalP program was used to detect a signal peptide sequence; it was used to determine whether CD52 encodes for a protein which is secreted through the secretory pathway via endoplasmic reticulum. CD52 has signal peptides in the first twenty amino acids at the N-terminus of the polypeptide [Figure 1]. The signal peptide will be cleaved at the 21st amino acid when the protein enters the lumen of endoplasmic reticulum.

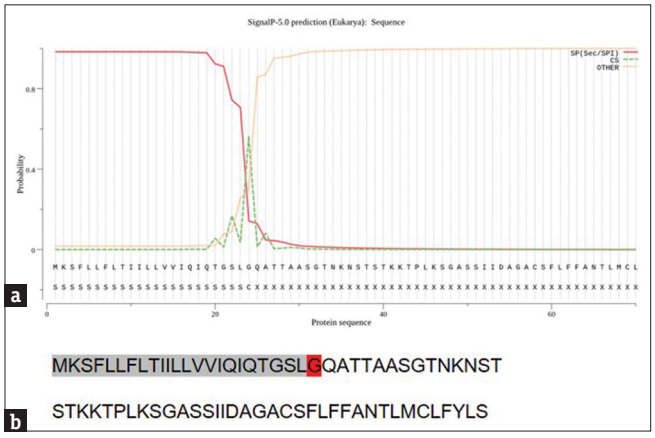

Figure 1: Analysis of CD52 signal peptides using SignalP5.0. (a) The red line represents signal peptides which span on the first 23 amino acids. The peak of broken green line represents a cleavage site which is located at amino acid number 24, whereas yellow line represents other (b) CD52 protein consists of 74 amino acids with signal peptides at the first 23 amino acids (grey shaded) and cleavage site at amino acid no 24 (red). The likelihood of the prediction is 0.98

This indicates that CD52 is a common secretory protein produced in the ribosome and subsequently transported into the lumen of the endoplasmic reticulum before it is distributed towards the cell membrane for secretion.

CD52 is highly expressed in the cauda epididymides

Tissue distribution analyses showed that CD52 was specifically expressed in the epididymis and showed the highest expression in the cauda region. Moderate expression was observed in the corpus. Besides in the epididymis, low expression was detected in the vas deferens. Expression in other tissues such as kidney, spleen, brain, muscle, heart, liver and intestine was undetected [Figure 2]. This result suggests that CD52 may have a certain role in the epididymis.

CD52 expression is regulated by androgen

One of the criteria of genes having male-reproductive-related functions is regulation by androgen. Experiment by removing the main source of androgen was carried out to examine whether CD52 was dependent on androgen. Our data indicate that there was a subtle increase in CD52 expression 6 h post castration [Figure 3]. The expression was subsequently decreased at day 1 after castration and dramatically downregulated on day 3 ($P < 0.05$). Dependency on androgen was confirmed when exogenous testosterone maintained the expression although not at a physiological level.

CD52 is regulated by lumicrine factors

We further investigated whether other factors in the lumen of efferent duct and epididymis other than androgen were involved in CD52 expression regulation. EDL showed CD52 was slightly upregulated at 6 h post EDL and decreased thereafter at days

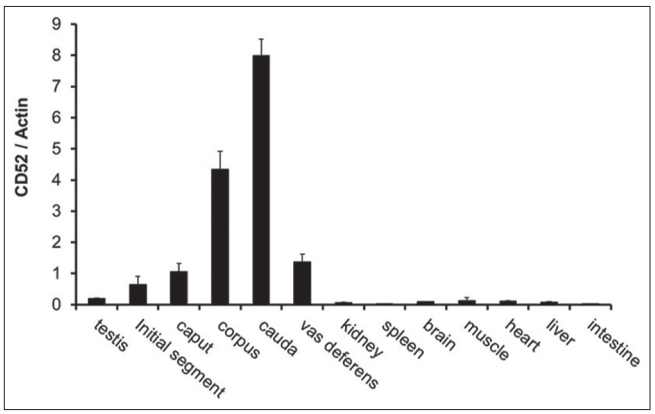

Figure 2: Quantitative real-time reverse transcriptase-polymerase chain reaction analysis of CD52 tissue distribution. On the x-axis, it shows total RNA from various tissues. The results showed that CD52 was exclusively expressed in the epididymis and the highest was in the cauda. The error bars indicate standard error of the mean ($n = 3$)

1–5 ($P < 0.05$) [Figure 4]. These data indicate that CD52 is also regulated by factors originating from the lumen (lumicrine).

CD52 is developmentally regulated

To examine whether CD52 expression is initiated when it is needed, expression in various developmental ages of mice was investigated. The results indicated that expression of CD52 started to increase at day 15 and continued to increase till day 40 before finally there was a sharp increase at day 60 postnatal age [Figure 5]. This experiment showed that the expression of CD52 was developmentally regulated.

Discussion

Sperm cells produced by the testis must undergo physiological and biochemical modifications to form motile and fertilization-competent cells. This process is known as sperm maturation that occurs via interactions with protein secreted by epididymal epithelium.[3,11] Genes involved in sperm maturation have criteria such as region-specific expression, secretory protein and regulated by endocrine and/or lumicrine factors.[12] We performed mRNA expression analyses of CD52 in mouse epididymis to investigate its putative involvement in maturation of sperm. Most of the proteins bathing the sperm cells during epididymal transit are secretory proteins produced by epithelial cells lining the epididymal duct. These proteins are synthesized in ribosomes with signal peptides which is subsequently cleaved when entering the lumen of the reticulum endoplasm. Our finding on CD52 signal peptide indicates that it constitutes secretory protein.

Our data demonstrated that CD52 was highly expressed in the cauda epididymis and a moderate level was found in the corpus region. Expression in other tissues was at background levels. This regional expression is one of the mechanisms of epididymis maturation in which a microenvironment is created and sperm undergoes maturation in a sequential order.[13] This is similar to several epididymal genes which are exclusively expressed in all regions of epididymis such as CRISP1[14] and cSrc.[15]

Androgen has been known to be the main regulator of epididymis functions; the current study showed that CD52 was affected by androgen presence being dramatically downregulated at day 1 to 3 days after castration and exogenous testosterone was able to prevent complete loss of expression. Testosterone replacement therapy restores cellular function in caput, corpus and cauda epididymis and increases epididymal gene expression.[16] Castration eliminates not only androgen but also testicular or lumicrine factors that are important to regulate gene expression in epididymis. This was also confirmed with EDL in which downregulation of CD52 was observed at day 1 of EDL.

The reason for a transient upregulation at 6 h post castration and EDL is not yet known; however, immune response such as TGF β1 is auto-inductive and may lead to increased growth factor expression at the site of injury.[17] CD52 regulation was in accordance with previous studies on epididymis-specific genes such as *Pate family*,[18] *Ep2*[19] and HongrES1. HongrES1 has been further developed as a candidate for male

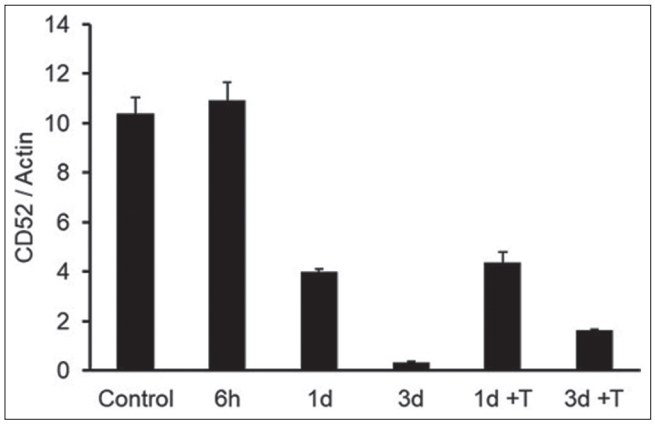

Figure 3: Androgen dependency analysis of CD52 using quantitative real-time polymerase chain reaction. Mice were castrated, and the cauda epididymides were isolated for RNA extraction after 6 h, 1 day and 3 day after gonadectomy (6 h G, 1 d G and 3 d G) and also 1 day and 3 day after castration with testosterone replacement therapy (1d+ T, 3d+ T). The results showed that CD52 was slightly upregulated at 6 h and significantly downregulated 1–3 days after castration ($P < 0.05$). The expression levels after testosterone injection were higher than mice day 1 and 3 castration without testosterone. Each level of expression was normalised against beta-actin. Error bars represent standard error of the mean ($n = 3$)

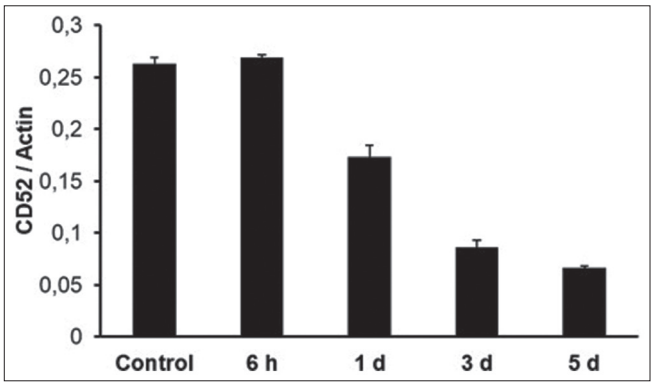

Figure 4: Efferent duct ligation (EDL) analysis using quantitative real-time polymerase chain reaction to examine the dependency of CD52 towards lumicrine factors in the epididymis. Mice efferent ducts were ligated for 6 h, 1 day, 3 day and 5 day after EDL and control (unligated). The result showed that CD52 was slightly upregulated at 6 h and significantly downregulated at day 1 to 5 ($P < 0.05$). Day 5 was the lowest level expression. Each level of expression was normalised to beta-actin. The error bars represent the standard error of the mean ($n = 3$)

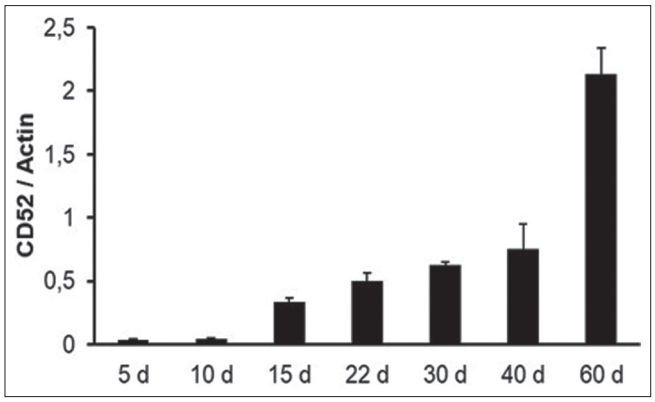

Figure 5: Postnatal expression analysis of CD52 using quantitative real-time polymerase chain reaction to examine whether developmental stages affect CD52 expression levels in the epididymis. Expression was examined at 5 day, 10 day, 15 day, 22 day, 30 day, 40 day and 60 day. Expression level for each group was normalised against beta-actin as a reference. Error bars represent standard error of the mean (standard error of the mean, $n = 3$). The result showed that expression of CD52 started at day 15 postnatal and was upregulated to day 60 postnatal

contraceptive agents due to its important role in sperm capacitation and male fertility.[6]

EDL eliminates lumicrine factors; one of those factors is ABP. ABP is important for binding testosterone that is produced by Leydig cell in the testis to enter the epididymis.[3,16] ABP deprivation by EDL can reduce testosterone entering the epididymis. Another example of lumicrine factor is FGF2 as a regulator of γ-glutamyl transpeptide.[20,21]

CD52 was detected at a low level at day 15 postnatally and increased dramatically on day 60. The greatest increase of mRNA in epididymis occurs during prepubertal period. Expression of CD52 increased in prepubertal period in line with increased testosterone to start spermatogenesis and may regulate epididymal epithelial differentiation into various cell types in mice aged 20 days.[22] Expression of epididymal genes increased during the prepubertal period also corresponds to cellular differentiation.[3] One of the epididymal genes that was developmentally regulated is 5α reductase. Expression of this gene was detected at day 21 and continuously upregulated at day 56 in *Mus musculus*.[23] This gene expression is similar to CD52 which is regulated by androgen and developmentally regulated.

Conclusions

CD52 is a secretory protein which is important for epididymal sperm maturation. The expression characteristic includes that it is only expressed in the epididymis, regulated by androgen and showed developmental regulation supporting a notion that CD52 may have a specific role in the epididymal sperm maturation. More detailed studies are required to validate the biological function of CD52 in the epididymis.

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Data availability statement

The authors need to mention where the data is available and that they are willing to share it upon reasonable request

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Nil.

Conflicts of interest

There are no conflicts of interest.

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