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Differential expression and antibacterial activity of WFDC10A in the monkey epididymis

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Abstract

The ability of the epididymis to perform its diverse functions stems from its regionalized gene and protein expression patterns. The differences in the gene expression patterns of the caput and cauda regions of the bonnet monkey epididymis were compared using the technique of differential display reverse transcriptase polymerase chain reaction. A transcript showing homology to human whey acidic protein 10 (hWFDC10A) was highly expressed in the monkey caput region. A peptide P2 was designed spanning a region of the monkey WFDC10A (mWFDC10A), which could inhibit the growth of gram-negative bacterial strains of *Escherichia coli*. P2 could permeabilize the bacterial cell membrane but was unable to permeabilize mammalian cells as evidenced by the lack of hemolysis upon incubation with the peptide. Expression of genes such as mWFDC10A may be essential in providing the first line of defense against microbial infections to the epididymal tract and thus rendering protection to the male gametes sheltered within the epididymis.

Keywords: Caput; Differential display; Hemolysis; Fluorescence; Innate immunity

1. Introduction

A singular feature that distinguishes the epididymis from other differentiated structures is its highly regionalized gene expression pattern. The segmentalized gene and protein expression culminates in a unique environment within the epididymal lumen that facilitates the process of sperm maturation. The spatial and temporal expression patterns are formed early on during development and are crucial for the functioning of the epididymis. A majority of studies have been carried out in the rodent epididymis to understand gene expression across the different regions of the epididymis (Jervis and Robaire, 2001; Hsia and Cornwall, 2004; Johnston et al., 2005). While these have provided a rich database of region-specific genes in the rodent, studies in the primate have been relatively few. It is necessary to analyze gene expression patterns in the epididymis of the higher primates, to obtain a better understanding of sperm maturation in the human. The non-human primate bonnet monkey (*Macaca radiata*) is a good model system due to its close similarity to human. Hence, employing the bonnet monkey epididymis, and using the technique of differential display reverse transcription polymerase chain reaction (DDRT-PCR), gene expression was compared in the caput and cauda regions of the epididymis. One differentially expressed transcript that showed very high expression in the monkey caput over cauda, was sequenced and found to have high homology to hWFDC10A.

Whey acidic proteins are characterized by the presence of 1 or 2 four-disulfide-core (FDC)-domains, each comprising of around 50 amino acids containing 8 cysteine residues in a conserved arrangement (Hennighausen and Sippel, 1982). A locus on human chromosome 20 was identified to possess a cluster of around 14 genes containing the FDC-domain, exemplified by human epididymal protein 4, eppin, WFDC10A and the small serine protease inhibitors such as elafin and secretory leucocyte protease inhibitor. Several of the FDC-domain containing proteins are capable of inhibiting microbial growth (McMichael et al., 2005). These proteins possess high basic amino acid content, by which they can penetrate the negatively charged bacterial cell wall. Interestingly, many of the FDC-domain containing proteins showed high expression in the epididymis (Clauss et al., 2002). These proteins, in addition to several others such as the

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defensins and cathelicidins are believed to form the first line of defense in the epididymis (Hall et al., 2002; von Horsten et al., 2002, 2004). Considering the role of the FDC-domain proteins in the innate immune system, the expression of mWFDC10A in the epididymis and its potential as an antibacterial agent was investigated.

2. Materials and methods

2.1. RNA isolation

Total RNA was extracted from the adult monkey caput and cauda regions, using TRIzol reagent (Sigma, St. Louis, MO, USA) which employs a convenient single-step liquid phase separation for isolation of RNA, DNA and protein (Chomczynski and Sacchi, 1987). All steps during isolation were performed according to manufacturer's instructions. The quantity of RNA was estimated spectrophotometrically at OD_{260}. An $OD_{260/280}$ ratio of 1.8–2 was indicative of a good quality RNA. Additionally, the integrity of the isolated RNA was checked on a 1% (w/v) MOPS–formaldehyde agarose gel. Total RNA was treated with 10 units of DNase I (Stratagene, La Jolla, CA) for 1 h at 37 °C. The enzyme was denatured at 65 °C for 15 min and the RNA was precipitated with three volumes of ethanol and 1/10th volume of 3 M sodium acetate, pH 5.2 at −70 °C for 1–2 h. The precipitated RNA was washed with 70% ethanol, air-dried, and the pellet was reconstituted in diethylpyrocarbonate-treated water and employed for reverse transcription.

2.2. Differential display reverse transcription polymerase chain reaction (DDRTPCR)

Total RNA free of DNA contamination was used for DDRTPCR as described by Linskens et al. (1998). In brief, 1 μg of RNA was allowed to anneal with 2.5 μM anchored reverse primer of sequence 5′-GCC CAA GCT TTT TTT TT CC-3′ at 70 °C, for 10 min. The reaction volume was adjusted to 10 μl with 200 μM dNTPs, reaction buffer and Moloney Murine Leukemia Virus reverse transcriptase. A control reaction lacking reverse transcriptase was included to confirm absence of non-specific amplification from genomic DNA. Following 1 h incubation at 37 °C, the enzyme was inactivated by heating at 95 °C for 5 min. The cDNA corresponding to 40 ng of RNA was subjected to PCR using 2.5 μM forward primer of sequence 5′-CGT GAA TTC GAG CCA GCG AA-3′ in presence of 2 μCi of α-^{32}P dCTP (3000 Ci/mmol) and 1 unit of *Taq* polymerase enzyme. The PCR condition involved four low stringency cycles (94 °C, 45 s; 41 °C, 60 s; 72 °C, 60 s) followed by 18 high stringency cycles (94 °C, 45 s; 60 °C, 45 s; 72 °C, 120 s) and primer extension at 72 °C for 10 min. The heat denatured PCR products were electrophoresed on a sequencing gel composed of 6% acrylamide and 48% urea at 1800–2000 V for 6 h in 1× TBE, until the xylene cyanol dye (used as loading dye) had run three quarters of the gel. The gels were dried onto Whatman 3M paper, marked with fluorescent papers for orientation, stapled to the X-ray film and exposed for 10–12 h at −70 °C. The X-ray film was developed until the DNA bands were visible on the film. The DNA bands that appeared to be differentially displayed in the autoradiograph were marked accurately and recovered from the gel. The DNA was purified using Qiagen gel extraction kit and subjected to sequencing. The sequence identity of the transcript was analyzed in the NCBI database.

2.3. Northern blot analysis

The differentially expressed fragment corresponding to mWFDC10A was PCR amplified and radiolabeled using Megaprime DNA labeling kit, as per the instructions provided by the manufacturer (Amersham Biosciences, UK). Twenty micrograms of total RNA from the monkey caput and cauda regions was size fractionated on a 1% MOPS–formaldehyde agarose gel, and transferred to a Nylon membrane in 20× sodium citrate–sodium chloride buffer, pH 7.2 for at least 24 h. The RNA blot was then subjected to pre-hybridization in Church buffer (Church and Gilbert, 1984). The blot was subsequently subjected to hybridization at 60 °C for 18 h in Church buffer containing the labeled mWFDC10A transcript (1–3 million counts/ml of hybridization solution). After hybridization, the blot was washed extensively and exposed to phosphor-imager screen for 2–4 h or autoradiographic film for 24–48 h and the film was developed until the bands appeared in the film. The labeled mWFDC10A probe was stripped-off the blot, and the blot was subjected to hybridization with labeled glyceraldehyde phosphate dehydrogenase (GAPDH) as probe, which was employed as internal control to assess equality of RNA loading.

2.4. Peptides for antibacterial assay

The expression of mWFDC10A in bacterial system led to accumulation of mWFDC10A in inclusion bodies and hence the ability of the mWFDC10A peptides to exhibit antimicrobial activity was assessed using mWFDC10A peptides. In order to test whether the mWFDC10A had antibacterial property, peptides with the sequence shown below, were designed using the program http://www.aps.unmc.edu/AP/main.php that calculates both charge and hydrophobicity of the peptides (Wang and Wang, 2004). The peptide was designed to have an isoelectric point (p*I*) as close to the native WFDC10A. Based on the preliminary secondary structure prediction of mWFDC10A (http://www.bmm.icnet.uk/servers/3djigsaw/), it was also observed that the P2 sequence was surface-exposed.

Sequence of the mWFDC10A peptides:

- P2: RPKLYLCKRFCESHRDCQANN
- P3: RPELYLCKRFCESDRDCQANN

Peptides were obtained from Genemed Inc., CA, USA and were at least 70% pure as assessed by their high performance liquid chromatography profiles (provided by the manufacturer). The peptide stocks were stored at −20 °C, while aliquots of the peptides were dissolved in the required buffer (10 mM sodium phosphate buffer pH 7.4) or water as required. The peptide P2 had p*I* 9.2 and a charge of +4 and hydrophobicity of 33%. The peptide P3 containing the two mutations (K–E; H–D, indicated by underline) had a p*I* of 6.1 and charge of '0'.

2.5. Kinetics of inhibition by mWFDC10A peptides

Two strains of the gram-negative bacterium *Escherichia coli* (*E. coli*), were chosen as targets for testing antibacterial activity. These strains were XL-1 blue MRF′ [supE44hsdR17recA1endA1gyrA46thirelA1lac$^-$Δ(mcrA)183 Δ(mcrCB-hsdSMR-mrr)173 F′[proAB$^+$lacI^{q}lacZΔM15Tn10 (tet^r)]] and RV [F$^-$ ΔlacX74thibglR°(Bgl$^-$)]. The bacterial strain XL-1 blue MRF′ was grown at 37 °C in Luria Bertini broth (LB) and an aliquot was streaked on plates comprised of LB containing 1.5% agar (LB-agar). A single colony was inoculated in 3 ml LB and grown overnight at 37 °C in an orbital shaker. One percent of the overnight culture was added to fresh medium and the bacterial cells were grown until mid-log phase (OD_{620} = 0.5–0.6). The bacterial suspension was diluted in LB medium to OD_{620} = 0.2, which corresponds to 5×10^7 CFU/ml. A working dilution of 5×10^6 CFU/ml was made and used for the bacterial assays. The assay was set up in duplicates and the procedure of Yenugu et al. (2003) was followed. In order to assess the dose dependent antibacterial activity of the peptides, working stocks of the bacterial cells XL-1 blue MRF′ and RV were prepared as described above and 100 μl aliquots from the stocks were incubated with peptide P2, at concentrations ranging from 10 to 300 μM. The cells were incubated at 37 °C in an orbital shaker for 30 min. As control, bacterial cells alone (in presence of 10 mM sodium phosphate buffer pH 7.4) were incubated similarly. In addition, a time-course study was performed using aliquots of 100 μl from the working stock of XL-1 blue MRF′ bacterial cell suspension, incubated in presence of 50 μM peptides P2 or P3 in an orbital shaker at 37 °C. As control, XL-1 blue MRF′ cells were incubated without the peptides (buffer alone), while as a non-specific protein control, incubation was carried out in presence of 50 μM bovine serum albumin (BSA). At 5, 10, 20 and 30 min, an aliquot was taken from the tubes, serially diluted in LB medium and plated on LB-agar plates. In case of both the experiments, the number of colonies was counted visually at the end of the incubation period and antibacterial activity was expressed as percentage survival, calculated as follows: (number of colonies

surviving after treatment with peptide or BSA/number of colonies in presence of buffer) × 100.

2.6. Outer membrane permeabilization assay

The ability of the peptides P2 and P3 to permeabilize the outer membrane of *E. coli* was assessed by a fluorescence based assay (Loh et al., 1984). An overnight culture of XL-1 blue MRF′ cells was grown to mid-log phase and washed twice with HEPES buffer (pH 7.2) containing 5 mM glucose and resuspended in the same buffer to an absorbance $OD_{620} = 0.4$. *N*-Phenyl-2-naphthylamine (NPN, Sigma, St. Louis, MO) was dissolved in acetone to a concentration of 0.5 mM. To the bacterial cell suspension, NPN was added to a final concentration of 10 μM and taken for fluorescence measurement. Partition of the fluorescent hydrophobic probe NPN into the cell envelope was monitored with an LS-50 fluorimeter (excitation, 350 nm; emission, 420 nm; slit width for both windows, 2.5 nm; 25 °C Perkin-Elmer Ltd., Beaconsfield, England); 100 μl of the NPN-bacterial cell suspension was monitored at the corresponding wavelengths until no change in fluorescence was observed. This was considered as base-line fluorescence. Peptides P2 and P3 were added at varying concentrations and the fluorescence was recorded as a function of time until no further increase in emission intensity was observed. Fluorescence of NPN alone and NPN with peptide, was measured to ensure that the change in fluorescence observed upon adding peptides to NPN-cell suspension was not due to non-specific interaction of NPN with the peptides. The fluorescence of the samples deducted from the base-line values was expressed as relative fluorescence units (RFU).

2.7. Hemolytic activity of the peptides

Hemolytic activity of the peptides was determined as described previously (Fernandez-Lopez et al., 2001). Fresh blood was collected from an adult rat in a heparin-coated tube, to prevent coagulation of the erythrocytes, and subjected to centrifugation at 400 × *g*, 5 min at 4 °C. The erythrocytes were washed twice in physiological saline (150 mM NaCl) and resuspended in the same to a final concentration of 5%. Hundred microlitres of aliquots were incubated with the peptides P2 and P3 ranging in concentration from 50 to 300 μM, for 2 h at 37 °C. As positive control, the erythrocytes were incubated with 1% Triton X-100, and erythrocytes in saline alone served as negative control. Following incubation, the suspensions were centrifuged at 400 × *g*, 5 min, at 4 °C. The supernatant was spectrophotometrically estimated at 560 nm. Hemolysis in presence of Triton X-100 and saline served as 100% and 0% hemolysis controls, respectively, and the percent hemolysis in presence of the peptides was calculated.

3. Results

3.1. Identification of WFDC10A by differential display RTPCR

RNA from the caput and cauda regions of the monkey epididymis was subjected to DDRTPCR analysis, and

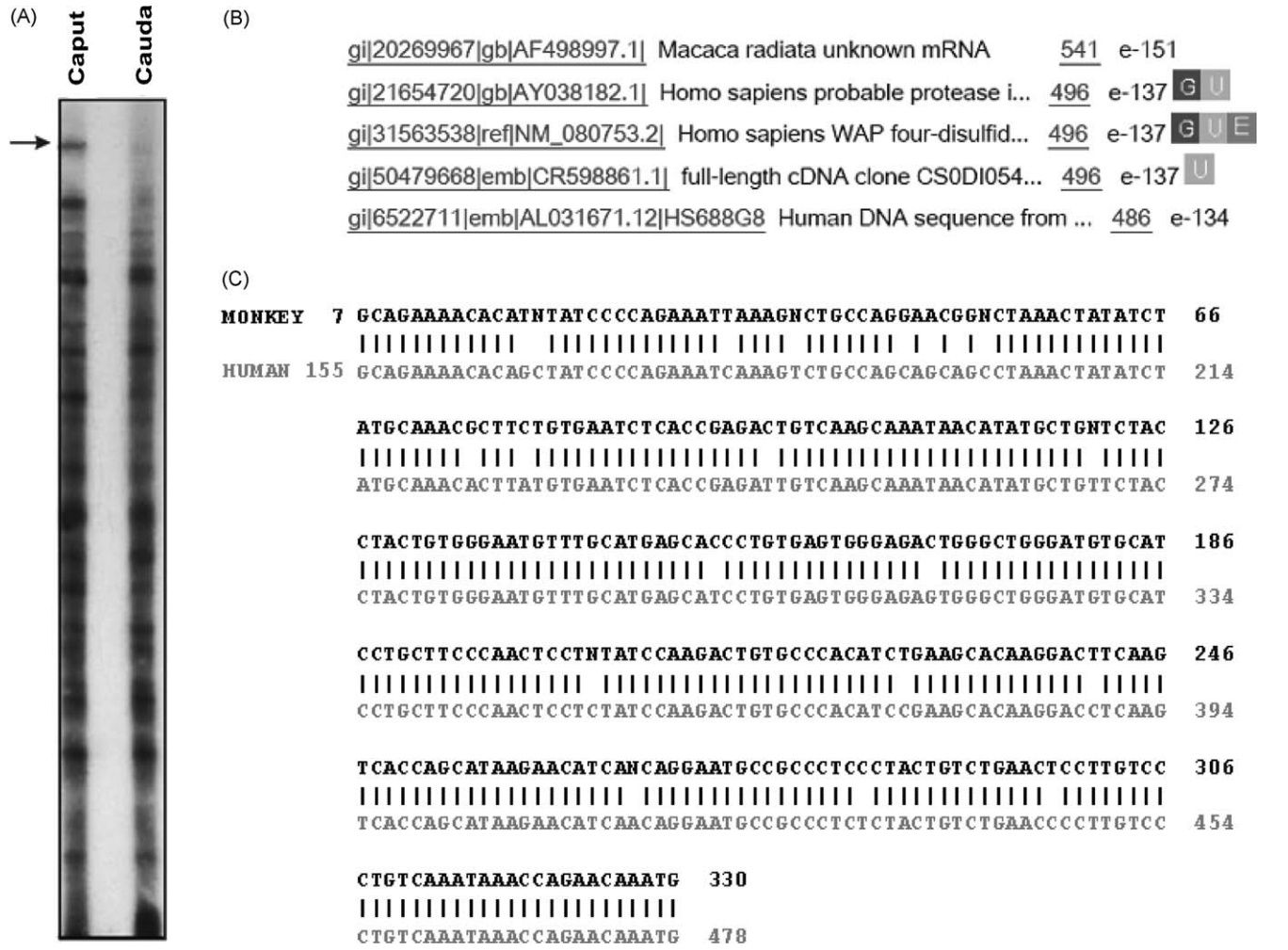

Fig. 1. Differential display analysis was performed with RNA from the monkey caput and cauda (A). Arrow in panel (A) indicates the transcript differentially expressed in the monkey caput compared to cauda. BLAST hits of the differentially expressed transcript showed maximum homology to hWFDC10A (B). The nucleotide sequences of mWFDC10A and hWFDC10A are depicted in panel (C).

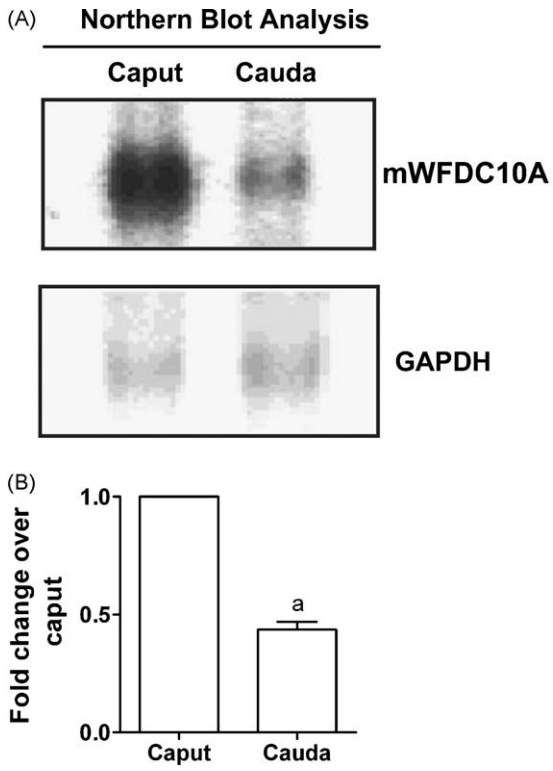

Fig. 2. The differential expression of mWFDC10A was confirmed by Northern blot analysis (A). The blot was stripped and probed with GAPDH as internal control. Histogram depicts the normalized signals of WFDC10A, expressed as fold change over caput (B). Data represents mean ± S.E.M. of three independent experiments: (a) $P < 0.05$.

the differentially expressed band is depicted by an arrow in Fig. 1A. The transcript showed higher expression in the monkey caput compared to cauda (Fig. 1A). The differentially expressed transcript was sequenced, and BLAST analysis (http://www.ncbi.nlm.nih.gov/BLAST) of the transcript revealed homology to hWFDC10A (Fig. 1B). The mWFDC10A mRNA sequence was submitted to NCBI database (accession no. AF498997). The sequence similarity between hWFDC10A and mWFDC10A is evident from Fig. 1C.

3.2. Validation of DDRTPCR by Northern blot analysis

The differential expression of mWFDC10A was confirmed by Northern blot analysis, employing RNA from the monkey caput and cauda regions and the mWFDC10A transcript as the labeled probe. A distinctly higher expression was observed in the monkey caput region in comparison to cauda, thus confirming the expression observed in differential display analysis (Fig. 2A and B).

3.3. Comparison of the hWFDC10A and mWFDC10A sequences

The translated human and monkey WFDC10A nucleotide sequences showed identical amino acids at most positions (Fig. 3A). The cysteine residues were at identical locations in both hWFDC10A and mWFDC10A, which is a signature of WFDC proteins. The p*I*'s of mWFDC10A and hWFDC10A were estimated and were found to be basic, 9.1 and 8.8, respectively, pointing to the cationic nature of these proteins (Fig. 3A). The disulfide bonds characteristic to the WFDC proteins are shown in Fig. 3B. An analysis of the hydrophobicity of mWFDC10A by Kyte-Doolittle method showed that the entire mWFDC10A was below the threshold of 1.8, together suggesting that mWFDC10A was cationic and hydrophilic (Fig. 3C). These observations and the known antibacterial activity of related FDC-domain proteins prompted us to analyze the ability of WFDC10A to kill bacteria.

3.4. Antibacterial activity of the peptides

The antibacterial activity of the peptide P2 was tested against two gram-negative *E. coli* bacterial strains, namely XL-1 blue MRF′ and RV using concentrations ranging from 10 to 300 μM with an incubation duration of 30 min (Fig. 4). While the P2 peptide was extremely active against XL-1 blue MRF′ cells, with an IC_{50} of 25 μM, the IC_{50} for strain RV was 50 μM. In order to further evaluate the time course of the antibacterial activity, *E. coli* strain XL-1 blue MRF′ was incubated in presence of 50 μM of the peptide P2 for a duration of 5–30 min. A drastic decline in bacterial survival was observed after 10 min incubation followed by overnight recovery (Fig. 5). Significantly, the peptide P3 or BSA (non-specific protein control, p*I* ∼ 6.1) failed to result in any reduction in bacterial colony numbers, consistent with the reported importance of the p*I* of the peptide/protein for antibacterial activity (Beers et al., 2002; Park et al., 2004).

3.5. Bacterial outer membrane permeabilization by P2 peptide

Many cationic peptides permeabilize the bacterial cell wall, disrupting the cell wall and causing leakage of cellular contents and lysis of bacteria. The ability of the peptides P2 and P3 to permeabilize the outer membrane of *E. coli* XL-1 blue MRF′ cells was evaluated using the NPN-fluorescence assay. Incubation of the peptide P2 with XL-1 blue cells resulted in a dose dependent increase in fluorescence within 5 min. In contrast, incubation with the peptide P3 caused negligible increase in fluorescence (Fig. 6).

3.6. Hemolysis of rat erythrocytes in presence of mWFDC10A peptides

The ability of the peptides to permeabilize the eukaryotic cell membrane was evaluated by incubation of rat erythrocytes in presence of increasing concentration of the two peptides for 2 h. As seen from Fig. 7, cell lysis was maximal in presence of the detergent Triton X-100, used as a positive control, while lysis in presence of saline was not detected. The peptide P2, even at the highest concentration of 300 μM caused minimal lysis at the end of 2 h of incubation and lysis in presence of P3 was not detected, suggesting that the peptide action was directed toward the bacterial membranes as opposed to mammalian cells.

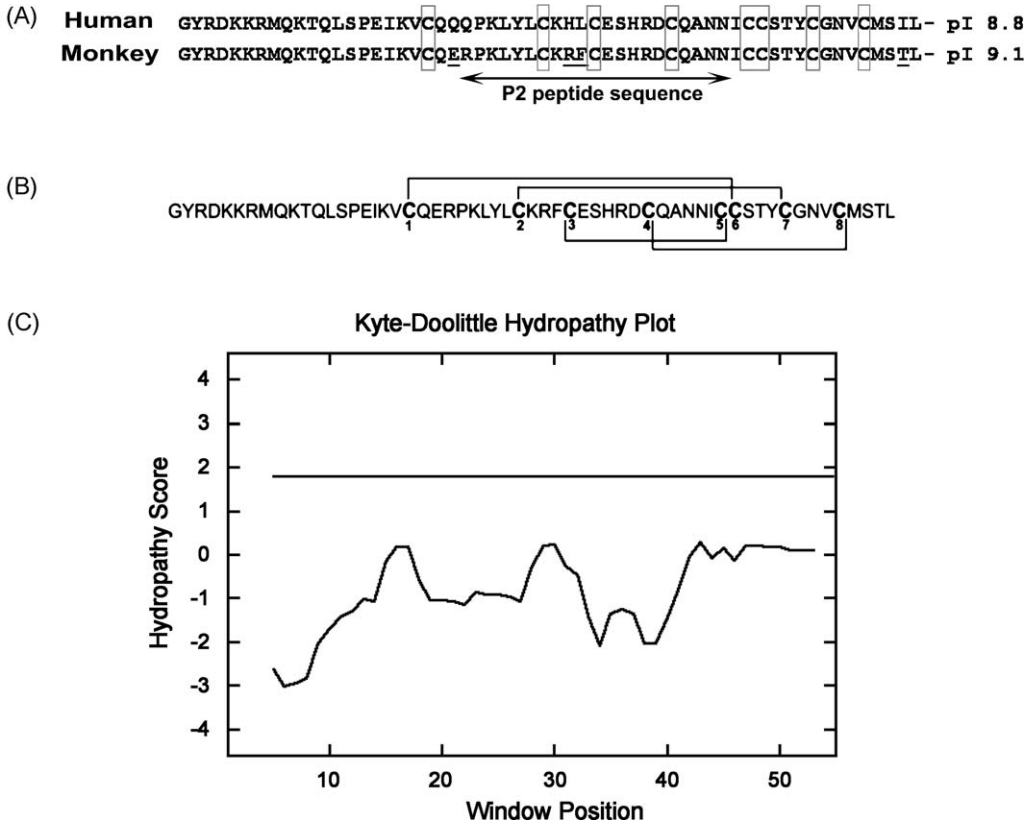

Fig. 3. The translated nucleotide sequences from hWFDC10A and mWFDC10A contain several identical amino acid residues, and both proteins have a basic p*I* (A). The underlined amino acids in the mWFDC10 sequence indicate the differences between hWFDC10A and mWFDC10A. The eight cysteine residues are indicated individually in box. The line between the arrowheads depicts the P2 peptide sequence. The protein sequence of mWFDC10A obtained from translation of nucleotide sequence contains the characteristic FDC-domain (B). The lines connecting the cysteines indicate the disulfide bonds, characteristic of the FDC-domain proteins. Kyte-Doolittle analysis of mWFDC10A shows the hydrophilic nature of mWFDC10A (C).

4. Discussion

As an organ that has evolved to perform multiple tasks including sperm maturation, the epididymis is crucial for regulating the concentration of luminal components, and for providing protection to the sperm while they are stored in the caudal region of the epididymis. Toward the goal of identifying the proteins that are involved in these functions, discovery-based methods have been employed to identify novel epididymal proteins or factors. Differential expression of mWFDC10A in the caput region of the epididymis is similar to the region-specific expression of other antibacterial proteins in the epididymis such as cathelicidins or defensins (Malm et al., 2000; Zaballos et al., 2004; Yenugu et

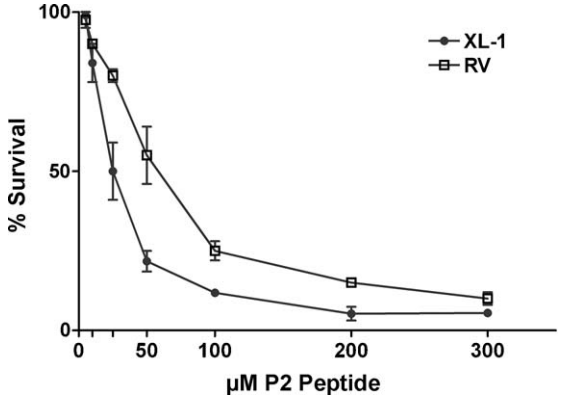

Fig. 4. *Escherichia coli* XL-1 blue MRF′ and RV cells were incubated with 10–300 μM concentration of the peptide P2 for 30 min. At the end of each incubation period, cells were diluted, streaked on LB-agar and incubated at 37 °C. As control, bacterial strains were incubated with buffer alone. The colonies surviving after treatment with P2 were expressed as percent survival after buffer only treatment. Data represents mean ± S.E.M. of three independent experiments.

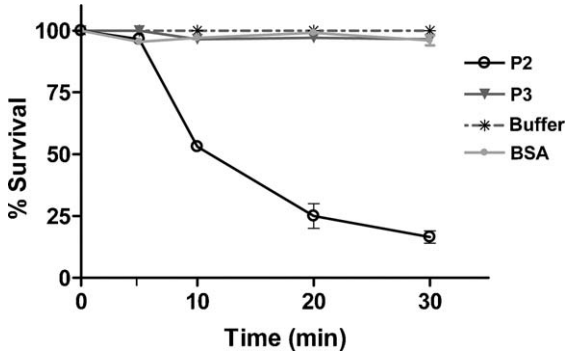

Fig. 5. XL-1 blue MRF′ cells were incubated with 50 μM of the peptides P2 and P3, BSA or buffer only for the indicated time durations. At the end of each incubation, cells were diluted, streaked on LB-agar and plates were incubated till colonies were visible. The number of colonies obtained after treatment with P2, P3 or BSA were expressed as percent survival after buffer only treatment. Data represents mean ± S.E.M. of four independent experiments.

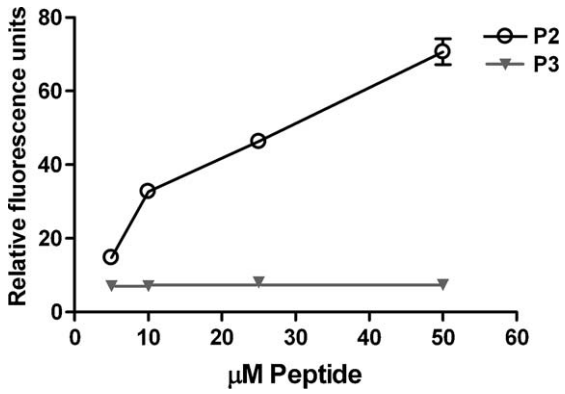

Fig. 6. XL-1 blue MRF′ cells were incubated with NPN dye and basal fluorescence was measured. Increasing concentration of the peptides P2 or P3, as indicated were added and fluorescence intensity was evaluated. The net fluorescence intensity after subtraction from basal fluorescence is depicted as relative fluorescence units. Data represents mean ± S.E.M. of three independent experiments.

al., 2006). These proteins are involved in innate immunity and our report suggests for the first time that mWFDC10A could be involved in epididymal defense.

Innate immunity may be particularly crucial to the immunologically privileged male reproductive tract where immunosuppressive proteins are produced and antibodies are partially excluded (Pollanen et al., 1988; Wyatt et al., 1988; Dettin et al., 2003). These mechanisms are in place to minimize the risk of auto-immune reaction to sperm, but at the same time reduce the adaptive immune response to invasion by pathogens. Innate immune proteins not only protect the male tract, but can form a protective armour on the sperm as they are carried into the female tract. Evolutionary conservation of this defense mechanism in rodents and primates suggests a fitness benefit. By protecting the sperm, these defense proteins perhaps also sustain fertilization competence and the survival of the species.

While, the reasons for the enriched expression of mWFDC10A in the caput are unclear, the epididymis is known to exhibit segment-specific expression of several genes and proteins, which uniquely contribute to the microenvironment within

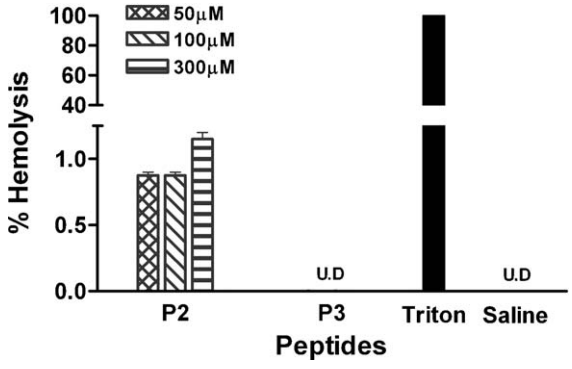

Fig. 7. Hemolytic ability of the peptides was tested using rat erythrocytes and 50, 100 and 300 μM of the P2 and P3 peptides. Hemolysis with 1% Triton X-100 was taken as positive control. Incubation of erythrocytes in presence of saline was taken as negative control. All incubations were carried out for 2 h and degree of hemolysis was evaluated at 560 nm. UD in the figure indicates undetected. Data represents mean ± S.E.M. of two independent experiments.

the epididymal tract (Krull et al., 1993; Zhang et al., 2006; Yuan et al., 2006). The property of high cationicity possessed by mWFDC10A and P2 peptide employed in the present study, is important in antibacterial proteins. P2 peptide inhibited growth of gram-negative bacterial strains XL-1 blue MRF′ and RV, although for reasons not clear at the moment, the inhibition was not to the same extent in both the strains. The differences in the peptide activity could be due to variation in the composition of LPS among bacterial strains (Gutsmann et al., 2005). However, this growth inhibition by P2 was charge dependent as the neutrally charged P3 peptide was unable to inhibit bacterial growth. In addition, the peptide P2 but not P3 could permeabilize the bacterial cells underlining the role of positive charge on the peptides in mediating their antibacterial function (Beers et al., 2002; Park et al., 2004). It has been observed for several peptides, as in this study, that abolishing the charge by mutation(s) can drastically reduce the antimicrobial activity of the peptides (Sitaram and Nagaraj, 1999). However, interestingly, the role of cysteines or disulfide bridges may be dispensable for antibacterial activity (Kluver et al., 2005). Our study and those of others indicate that electrostatic interactions play a predominant role in determining antibacterial activity of the peptide or protein, especially toward gram-negative strains while altering structural properties seem to have a more pronounced effect on hemolytic abilities (Dathe et al., 1997; Kluver et al., 2005). While it may be argued that the antibacterial activity of a partial peptide sequence such as P2 may not reflect the in vivo function of the native WFDC10A, given the similar p*I*'s of P2 and mWFDC10A, and the antibacterial activities of other WFDC proteins such as SWAM1 and 2 and eppin (Hagiwara et al., 2003; Yenugu et al., 2004), it is likely that WFDC10A may have a similar function.

Interestingly, unlike the bacterial membranes which could be permeabilized by P2, the peptide was unable to permeabilize rat erythrocytes as evidenced by the lack of hemolysis upon incubation with the peptide. Thus, while the high positive charge on P2 can mediate interaction with the negatively charged bacterial membrane, a low hydrophobicity ensures minimal interaction with the neutrally charged mammalian cell membranes. The intrinsic differences in membrane composition determine the vulnerability of bacterial and mammalian membranes to the cationic antimicrobial proteins, and provide an in-built mechanism to prevent injury to the "self-membranes" (Glukhov et al., 2005). The ability of P2 to kill bacteria by membrane permeabilization does not exclude the possibility of mechanisms involving disruption of macromolecular synthesis inside the bacteria as seen for other antimicrobial peptides (Park et al., 1998; Brown and Hancock, 2006). In addition, homology of mWFDC10A to serine protease inhibitors may enable it to inhibit exogenous proteases secreted by pathogens as suggested for other members of the WFDC family, a possibility that needs to be investigated for mWFDC10A.

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