

RESEARCH ARTICLE

Open Access



In vitro chemokine (C-C motif) receptor 6-dependent non-inflammatory chemotaxis during spermatogenesis

Ruiying Diao¹, Xueyong Cai¹, Lu Liu¹, Lihua Yang², YongGang Duan¹, Zhiming Cai¹, Yaoting Gui² and Lisha Mou^{1*}

Abstract

Background: Chemokine (C-C motif) receptor 6 (CCR6) is present in sperm and plays a significant role in sperm motility and chemotaxis acting in the reproductive tracts. However, the expression and functional significance of CCR6 in testis are still poorly understood, especially in the process of spermatogenesis.

Methods and results: CCR6 was expressed in spermatogenic cell lines and its expression was shown in an age-dependent upregulation manner from puberty to adulthood in mouse testis. Immunostaining results confirmed the localization of CCR6 in testis. Further chemotaxis assays demonstrated that spermatogenic cells GC-1 and -2 exhibited a directional movement toward CCR6-specific ligand such as CCL20 or Sertoli cells in vitro.

Conclusions: The present findings indicate that CCR6 is involved in the chemotaxis of spermatogenic cells in vitro and promotes chemotaxis under non-inflammatory conditions during normal spermatogenesis.

Keywords: CCR6, CCL20, Chemotaxis, Sertoli cell, Spermatogenesis

Background

In the seminiferous epithelium, each Sertoli cell supports at least 50 germ cells and plays a crucial role during spermatogenesis. Conceivably, spermatogenesis process is involved with extensive junction restructuring particularly at the blood-testis barrier (BTB) that occurs at stages VII–VIII of seminiferous epithelial cycle [1]. Specifically, spermatogenesis is rigorously controlled by endocrine hormones and local environment. For local control, some cytokines and/or chemokines act as growth and differentiation factors in seminiferous tubule under normal physiological conditions as well as during pathophysiological states. For example, cytokines have been shown to modulate Sertoli cell transferrin release, indicating a cytokine-mediated bidirectional communication between testicular cells [2]. Evidence has shown

that some cytokines and/or chemokines can potentially exert certain effects on cell populations involved in spermatogenesis and influence the post-meiotic stages of spermatogenesis [3]. For instance, rescue assays using recombinant chemokines indicated that C-C motif ligand 9 facilitates Sertoli cell chemoattraction of stem/progenitor spermatogonia, which express C-C receptor type 1 [4].

Chemokine (C-C motif) receptor 6 (CCR6) is a chemokine involved in cell interactions and leukocyte chemoattraction. It can recruit immunocytes into inflammatory tissues induced by its specific ligand chemokine (C-C motif) ligand 20, CCL20 [5]. Recent evidence indicates that CCR6 is expressed in the tail and post-acrosomal region of sperm as well as in the mid-piece and neck [6]. Appreciably, β -defensin1 (DEFB1), another ligand of CCR6, is a specific secretory polypeptide from the epididymis [6, 7]. In the testis, testicular cells can produce and secrete relatively low levels of DEFB1, which may be involved in the maintenance of sperm during the epididymis-vas deferens pass [8]. Furthermore, CCR6

*Correspondence: molly__molly@163.com

¹ Reproductive Medicine Centre, Shenzhen No. 2 Hospital, Shenzhen University School of Medicine, No. 3002, Sunguang West Road, Shenzhen 518035, Guangdong, China

Full list of author information is available at the end of the article



may be involved in motility changes of sperm related to capacitation process, particularly in kinetic alterations and chemotaxis. Functional experiments also illuminate that CCR6 in sperm triggers Ca^{2+} mobilization stimulated by DEFB1 and CCL20, resulting in forward motility and chemotaxis of sperm in the reproductive tracts [9]. However, little is known about the constitutive expression and function of CCR6 in the testis.

Methods

Reagents

Two rabbit anti-CCR6 antibodies were purchased from Abcam company (ab78429, USA) and Sigma (SAB2702036, USA), respectively. Rabbit normal IgG (sc-2027), mouse monoclonal anti-occludin antibody (sc-133256) and anti- β -Tubulin antibody (sc-5274) were obtained from Santa Cruz Biotechnology (CA, USA). Recombinant Human CCL20/MIP-3 α (rCCL20) was from R&D System (360-MP/CF, Minneapolis, MN, USA). Enzyme-linked immunosorbent assay (ELISA) for CCL20 was obtained from Cloud-Clone Corp. (SEA095Hu, USA). Recombinant human active DEFB1 (rDEFB1) was purchased from Abcam (ab50048).

Cell cultures

The established lines of germ cell-1 spg (GC-1), germ cell-2 spc (GC-2), Sertoli cell origin TM4 and 15P-1, were purchased from the American Type Culture Collection (Rockville, MD, USA). GC-1 cells are characterized as being in a stage between mouse spermatocytes and spermatogonia, whereas GC-2 cells express several markers characteristic of mouse meiotic spermatocytes [10, 11]. GC-1 and -2 were cultured at 37 °C in a 5% CO_2 atmosphere in Dulbecco's modified Eagle's media (11965-092; Life Technologies, Carlsbad, CA, USA) containing L-glutamine, 110 mg/L sodium pyruvate, and pyridoxine hydrochloride supplemented with 10% fetal bovine serum and 100 U/mL penicillin–streptomycin. TM4 cells were cultured at 37 °C with 5% CO_2 in DMEM supplemented with 10% FBS, and 0.5% penicillin–streptomycin (P/S). 15P-1 cells were cultured at 32 °C with 5% CO_2 in DMEM supplemented with 5% FBS, 1% sodium pyruvate, and 0.5% P/S.

Primary Sertoli cells (SC) were isolated from 20-day-old male mice testes and cultured in serum-free DMEM/F12 medium supplemented with EGF (2.5 ng/mL), bovine insulin (10 $\mu\text{g}/\text{mL}$), human transferrin (5 $\mu\text{g}/\text{mL}$), bacitracin (5 $\mu\text{g}/\text{mL}$) and gentamicin (20 $\mu\text{g}/\text{mL}$) at 35 °C with a humidified atmosphere of 5% CO_2 in air [12]. After 1 week of culture, cell purity was established through cell morphology after vimentin immunostaining, presence of lipid inclusions active phagocytosis, and the absence of Leydig cells.

Samples

The use of human specimens for this project was approved by the Regional Committee for Medical Research Ethics and the Human Ethics Committee of Shenzhen No. 2 Hospital and Peking University Shenzhen Hospital. Informed consent was obtained from each patient. The approval reference number is 20090018. The fertile human testis specimens were obtained from post-mortem studies and orchiectomies. The specimens were andrologically examined to exclude testicular tumors by testicular ultrasound. The age range of the subjects was 24–40 years old. All of the fertile men had fathered at least one child without assisted reproductive techniques, such as IVE, ICSI, and IMSI. Men with a history of orchitis, surgery of the vasa deferentia, bilateral orchiectomy, chemotherapy or radiotherapy, obstructive azoospermia (OA), non-obstructive azoospermia (NOA), retrograde ejaculation, bilateral cryptorchidism, numerical or structural chromosome abnormalities, or Y chromosome deletions were excluded.

Male C57/6BL mice (aged 3 days, 2, 4 or 8 weeks) were purchased from the Southern Medical University Animal Center, Guangzhou, China. Animals were killed by CO_2 asphyxiation. All animals were treated according to the Guide for the Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources for the National Research Council. And the study was approved by the local ethical committee.

Western blot analysis

Lysed proteins from GC-1 or -2 cell lines and testis tissues were centrifuged for 10 min at 12,000 $\times g$. The supernatant fractions were mixed with 5 \times SDS sample buffer and boiled for 5 min. Each extract containing 40 μg proteins was subjected to 10% polyacrylamide gel electrophoresis and transferred onto polyvinylidene difluoride membranes (Millipore, Billerica, MA, USA). Membranes were blocked with Tris-Buffered Saline Tween-20 (TBST, pH7.5) containing 5% nonfat dry milk or 1% BSA for 3 h. Then, it was incubated overnight at 4 °C with respective antibody for CCR6 or β -tubulin, followed by corresponding HRP-conjugated secondary antibodies for 2 h at room temperature. Bound antibodies were detected using the chemiluminescence Western blot detection kit (Pierce, Waltham, MA, USA) according to the manufacturer's recommendations. Band intensities were analyzed with the Image J software (NIH, Bethesda, MD, USA).

Immunohistochemistry and immunofluorescence staining

Testicular tissues obtained from testis tissues were immediately stored in 4% paraformaldehyde solution after orchiectomy. After fixing and embedding, serial sections

of 5 μm thickness were obtained. Paraffin sections of testis were deparaffinized using xylene, rehydrated through a graded series of ethanol, and incubated in 3% hydrogen peroxide in methanol. After antigen retrieval and blocking with 5% normal goat serum, sections were incubated with rabbit anti-CCR6 or anti-TNF- α antibody overnight at 4 $^{\circ}\text{C}$, followed by biotin-conjugated anti-rabbit secondary antibody. DAB reaction was developed at the site of peroxidase activity. Then, sections were rinsed and washed with deionized water for 5 min, and the nuclei were counterstained with Mayer's hematoxylin and mounted. For negative control, the primary antibody was replaced with goat serum without peptide immunization. The specimens were observed under a microscope (DMLB; Leica Microsystems, Wetzlar, Germany).

For immunofluorescence staining, sections from testes were washed three times after overnight incubation with rabbit anti-CCR6 antibody or and mouse anti-occludin antibody overnight at 4 $^{\circ}\text{C}$. Slides were incubated with Alexa 488-conjugated goat-anti-rabbit or plus Alexa 594-conjugated goat-anti-mouse secondary antibody (1:500 dilution, Molecular Probes, Eugene, OR) at room temperature for 1 h. Under a fluorescence microscope (SPOT software, Olympus, Tokyo, Japan), ten random fields were captured for statistical analysis. Negative controls were obtained after omitting the primary antibody.

ELISA for CCL20

Quantitative measurement of CCL20 level was analyzed in the culture supernatant from TM4, 15P-1 cell line or primary Sertoli cells (SC) with an ELISA-based kit following manufacturer's instructions. The culture supernatant was centrifuged and separated at $1000\times g$ for 20 min. Transwells were used for the diluted standard, blank, and sample. The luminescent signal produced from TMB substrate was measured at 450 nm using a spectrophotometer (Thermo Fisher, Waltham, MA, USA). Each sample was tested in duplicate in two separate measurements.

Chemotaxis assays

Chemotaxis assays were performed using 24-well transwells (6.5 mm/diameter, 8 μm /pore; Costar, Corning Inc., Corning, NY, USA) as previously described [13]. Briefly, GC-1 or -2 (1×10^4 /cells, 100 μL) were added to 8 μm -pore transwell inserts, coated with 25 μg of growth factor-depleted Matrigel (Becton–Dickinson Immunocytometry Systems, San Jose, CA, USA). The bottom chamber contained serum-free RPMI with or without CCL20 (50 ng/mL) or rDEFB1 (500 ng/mL) or TM4 or 15P-1 cell line or primary SC (1×10^4 /cells, 100 μL). For the purpose of blocking migration, each condition was prepared in a separate aliquot and incubated with anti-CCR6 antibody (ab78429 or SAB2702036). Normal rabbit IgG

was used as negative control. Migration was conducted at 37 $^{\circ}\text{C}$, 5% CO_2 for 24 h. Migrated cells were collected from the lower compartment, centrifuged at $450\times g$, and counted using a hemocytometer. Only live cells, as determined by trypan blue exclusion, were considered in the quantification. Experiments were conducted in triplicate, and results were shown as the number of cells migrated in response to each treatment, respectively. The results for transwell assays were reported as chemotactic index (fold increase over baseline).

Statistical analysis

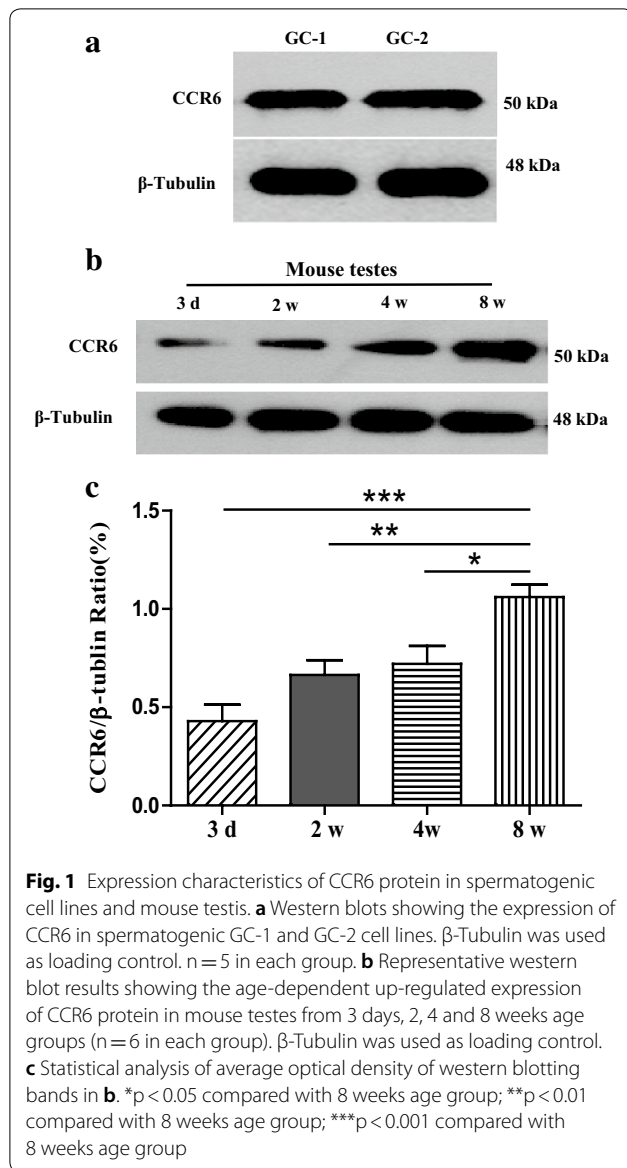
The Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. All experiments were conducted at least three times. Data were expressed as the mean \pm SEM. Comparisons were subjected to one-way analysis of variance (for multi-group comparisons). Statistical significance was set at $p<0.05$.

Results

Expression characterization of CCR6 and CCL20 in normal testis

To investigate whether CCR6 protein was present in testis and plays an important role during spermatogenesis, the study investigated the expression of CCR6 in spermatogenic cell lines and seminiferous epithelium, respectively. Firstly, western blot results showed that CCR6 was expressed in mouse spermatogonia GC-1 and spermatocyte GC-2 cell lines (Fig. 1a). Then, we compared the expression of CCR6 in mouse testes obtained from different age groups. As shown in Fig. 1b, c, CCR6 expression was significantly up-regulated by 59.53% ($p<0.001$), 37.41% ($p<0.01$) and 32.08% ($p<0.05$) in adult mice (8 weeks age group, 1.06 ± 0.20) compared to 3 days, 2 and 4 weeks age groups, respectively. Furthermore, the presence of CCR6 expression was also confirmed in normal adult human testis (Additional file 1: Figure S1). Therefore, the presence of CCR6 protein in testis and its expression in an age-dependent up-regulation manner may indicate the coincidence between the spermatogenesis and CCR6 expression in the testis from puberty to adulthood.

Under normal physiological states, focal inflammatory cytokines can be found in the testicular interstitium, such as TNF- α (Additional file 2: Figure S2). However, no inflammatory infiltration exists in the seminiferous epithelium due to the integrity of blood-testis barrier (BTB). Then, immunostaining data revealed that CCR6-positive signals were detected in the spermatogenic cells, spermatids and testicular interstitial area of mouse and human testes (Fig. 2a, b), respectively. The co-localization of CCR6 and occludin, a key member of tight



junction strands of blood-testis barrier (BTB), suggested that CCR6 signals were localized in the cell membrane, especially in the tight junction between Sertoli and germ cells (Fig. 2c). Then, this study attempted to determine the cellular origin of CCR6-specific ligand CCL20 in the

seminiferous epithelium. The data from ELISA results confirmed that CCL20 was present in TM4, 15P-1 cell line and primary SC (Fig. 3), indicating that Sertoli cells may be the main cellular origin of CCL20.

Involvement of CCR6 in the non-inflammatory chemotaxis during normal spermatogenesis

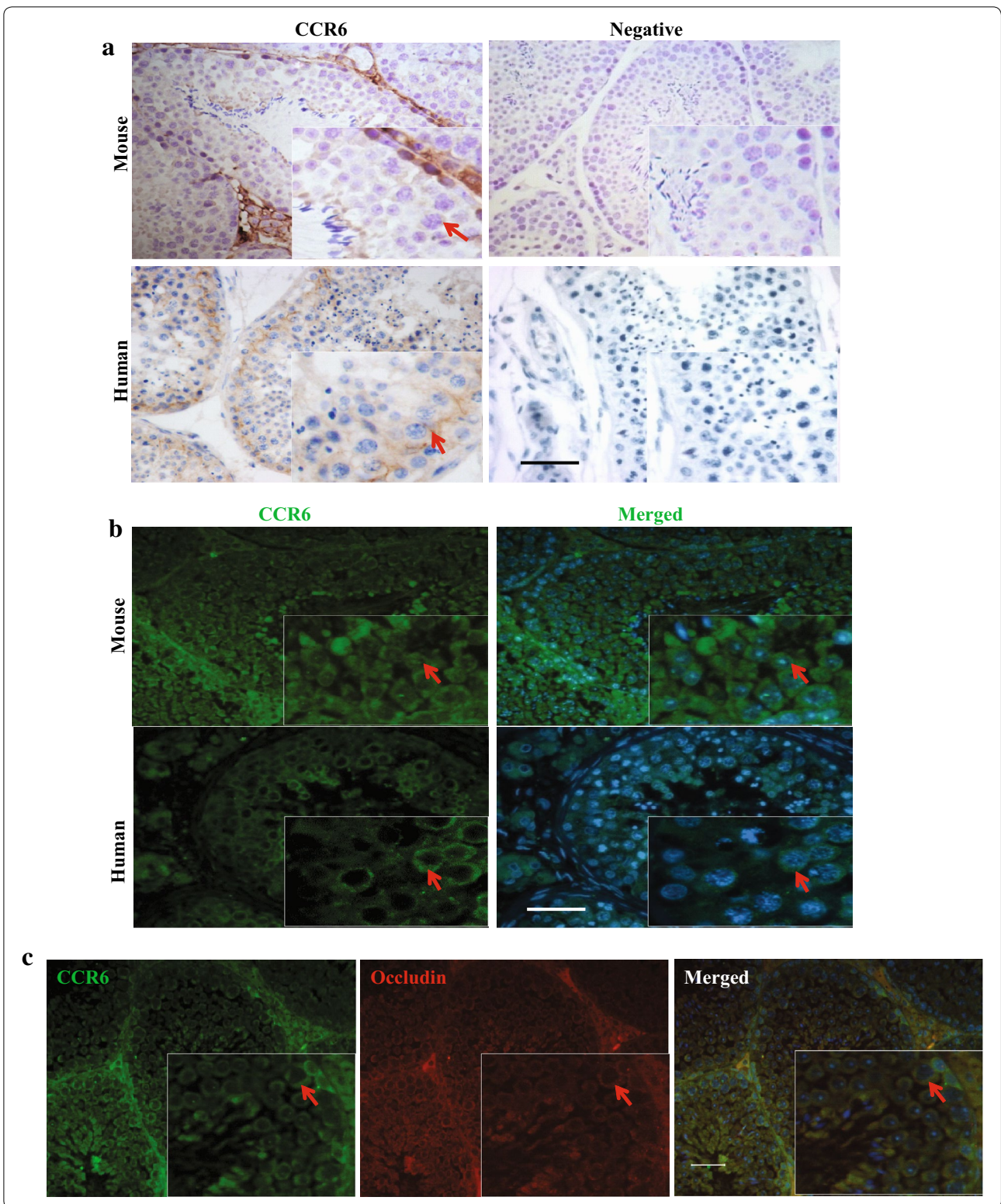
To investigate whether CCR6 is involved in the chemotaxis of spermatogenic cells, this study used rCCL20 or culture supernatant from TM4 or 15P-1 cell line as stimulus factors, respectively. The chemotaxis assay results showed that rCCL20 can significantly stimulate the chemotaxis of two cells (Fig. 4a, b). The immunodepletion of CCR6 by specific anti-CCR6 antibody (two antibodies from different company) significantly impaired cell chemotaxis compared with non-specific normal IgG, indicating that chemotaxis induction of rCCL20 was dependent on the function of CCR6 receptor on GC-1 or -2. Using conditional co-culture, chemotaxis induction by the culture supernatant from TM4 cell line can mimic the effects of rCCL20. The antagonistic effect of anti-CCR6 antibody could also be observed in this TM4 or 15P-1 cell line supernatant-induced chemotaxis acting (Fig. 4c, d). Furthermore, CCR6-dependent non-inflammatory chemotaxis was also found under the stimulation by rDEFB1 or culture supernatant from primary SC (Additional file 3: Figure S3). Therefore, these in vitro functional results further confirmed that CCR6 may play an important role in the chemotaxis of spermatogenic cells. That is, Sertoli cells produced CCL20, which can induce GC-1 or -2 non-inflammatory chemotaxis through the receptor CCR6.

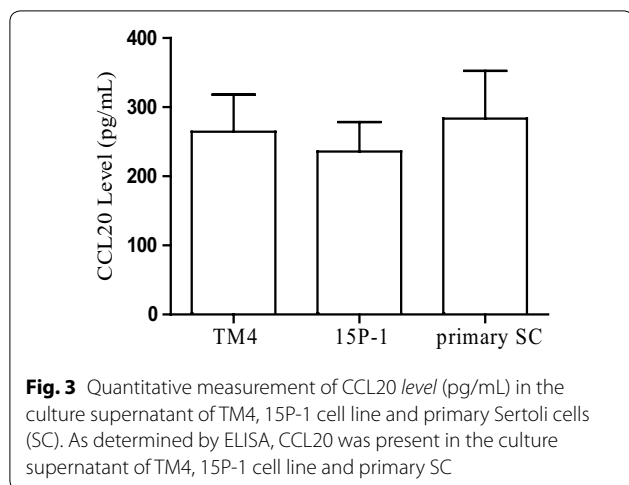
Discussion

Spermatogenesis is a complex process mediated by local growth or differentiation factors and a compartmentalized process sequestered within the BTB [14, 15]. Increasing amounts of evidence indicates the non-inflammatory roles of growth factor and cytokines in development and differentiation of germ cells during spermatogenesis [16, 17]. Sertoli cells are involved in the transduction of signals using testicular receptor to regulatory elements of several responsive genes under non-inflammatory autocrine/paracrine states [16]. Previous

(See figure on next page.)

Fig. 2 Characteristics of CCR6 localization in normal testis. **a** Representative immunohistochemical images showing the expression of CCR6 in mouse and human testes (arrow). Nuclei were counterstained with hematoxylin. Enlarged images are shown in the inset; at least three independent experiments were done. Scale bar = 50 μ m. **b** Representative immunofluorescence images showing the expression of CCR6 in mouse and human testes (arrow), respectively. Nuclei were counterstained with Hoechst 33258. Enlarged images are shown in the inset; At least three independent experiments were done. Scale bar = 50 μ m. **c** Representative co-staining images showing the expressions of CCR6 (green) and occludin (red) in mouse testis (arrow). Nuclei were counterstained with Hoechst 33258. Enlarged images are shown in the inset; At least three independent experiments were done. Scale bar = 50 μ m





studies reported that chemotaxis acting on sperm after ejaculation from the epididymis depends on CCR6 in sperm, primarily improving forward motility and promoting sperm-egg fertilization [6, 9]. CCR6 in human and mouse sperm mostly originates from spermatogenic cells, although it is possible that CCR6 is contributed to the sperm by epididymosomes [18, 19]. Recent research has also pointed to a putative role for CCR6/CCL20 axis in optimal germinal centre (GC) kinetics and B memory cell function [20, 21]. Then, is CCR6, as the receptor of CCL20, involved in the process of spermatogenesis?

As for the presence of CCR6 in testis, mouse *Ccr6* mRNA was previously reported to express in testis [6]. In the present study, it was further confirmed by the presence of CCR6 proteins both in spermatogenic cell lines and testis tissues. Then, we found that CCR6 was mainly expressed and localized in spermatogenic cells, such as spermatogonia, spermatocytes and spermatid cells. Furthermore, CCR6 signals were also detected in the tight junctions. After confirming the presence of CCR6, the ELISA results elucidated that CCL20 was present in the culture supernatant of TM4, 15P-1 cell line and primary SC. At the same time, the age-dependent up-regulation of CCR6 expression in mouse testis from puberty to adulthood, indicated the coincidence between CCR6 expression and essential role of CCR6 chemotaxis during normal mouse spermatogenesis.

In the seminiferous epithelium, non-lymphoid cells such as Sertoli cells, which can secrete cytokines, may be involved in germ cell-germ cell interactions in a stage-specific manner during spermatogenesis [22]. For example, IL-6, which is produced by Sertoli cells [23], is a stage-specific paracrine regulator of the seminiferous epithelium exerting specific inhibitory action on meiotic DNA synthesis [24]. Accordingly, the local

chemokine cycles are important for spermatogenesis [18]. Then, what is the role of CCL20-CCR6 in spermatogenesis? Previous studies showed that CCL20 is involved in the non-inflammatory chemotaxis of sperm; thus, the present study focused on this during spermatogenesis. The *in vitro* chemotaxis experiments indicated that chemotaxis of CCL20 (50 ng/mL) may be involved in the migration of GC-1 and -2, and this process can be significantly inhibited by CCR6-specific antibody. Using two CCR6-specific antibodies from different commercial companies, this antagonistic effect can be achieved. At the same time, Sertoli cell-conditioned medium can induce the non-inflammatory chemotaxis of GC-1 and -2, which can be obviously inhibited by CCR6-specific antibody. This finding indicates that non-inflammatory chemotaxis of GC-1 and -2 induced by Sertoli cell-conditioned medium also needs the participation of CCR6 in germ cells. The further results also suggest that the migration of GC-1 and -2 can be stimulated by rDEFB1, which can also be significantly inhibited by CCR6-specific antibody (Additional file 3: Figure S3). Therefore, we provide evidence that the involvement of Sertoli cells and CCR6-it ligands (CCL20 or DEF1) interaction in the migration of spermatogenic cells which is a relevant process during spermatogenesis.

Accordingly, Sertoli cells provide germ cells with the appropriate mitogens, differentiation factors, and energy sources, as well as protect them from harmful agents and from the host's own immune system. Specifically, chemokine ligands via their receptors were required for the maintenance of mouse spermatogonial stem cells. For example, CXCL12-CXCR4 signaling is required for the maintenance of mouse spermatogonial stem cells [25]. Macrophage inflammatory protein-1 alpha is a regulator of mitotic and meiotic DNA synthesis during spermatogenesis [26]. A previous study demonstrated that CCL20/CCR6 are involved in the metastasis of a variety of tumors, including prostate, colorectal and lung cancer [27, 28]. As determined by protein chip analysis, several chemokines (including some that act through CCR6, such as CCL20 and protein hormones) were present in human follicular fluid, endometrial secretions, and seminal plasma. CCL20/CCR6 interactions can lead to proliferation and migration dependent in part on extracellular signal-regulated kinase (ERK) phosphorylation during lung adenocarcinoma growth [28]. *In vitro* Sertoli cells can produce and secrete CCL20. In this study, the chemotaxis assays demonstrated that spermatogenic GC-1 and -2 cell lines exhibited a directional movement toward CCL20 or Sertoli cells *in vitro*. This *in vitro* study using GC-1 and -2 cell lines confirmed that CCR6 was involved in the non-inflammatory chemotaxis of germ cells when

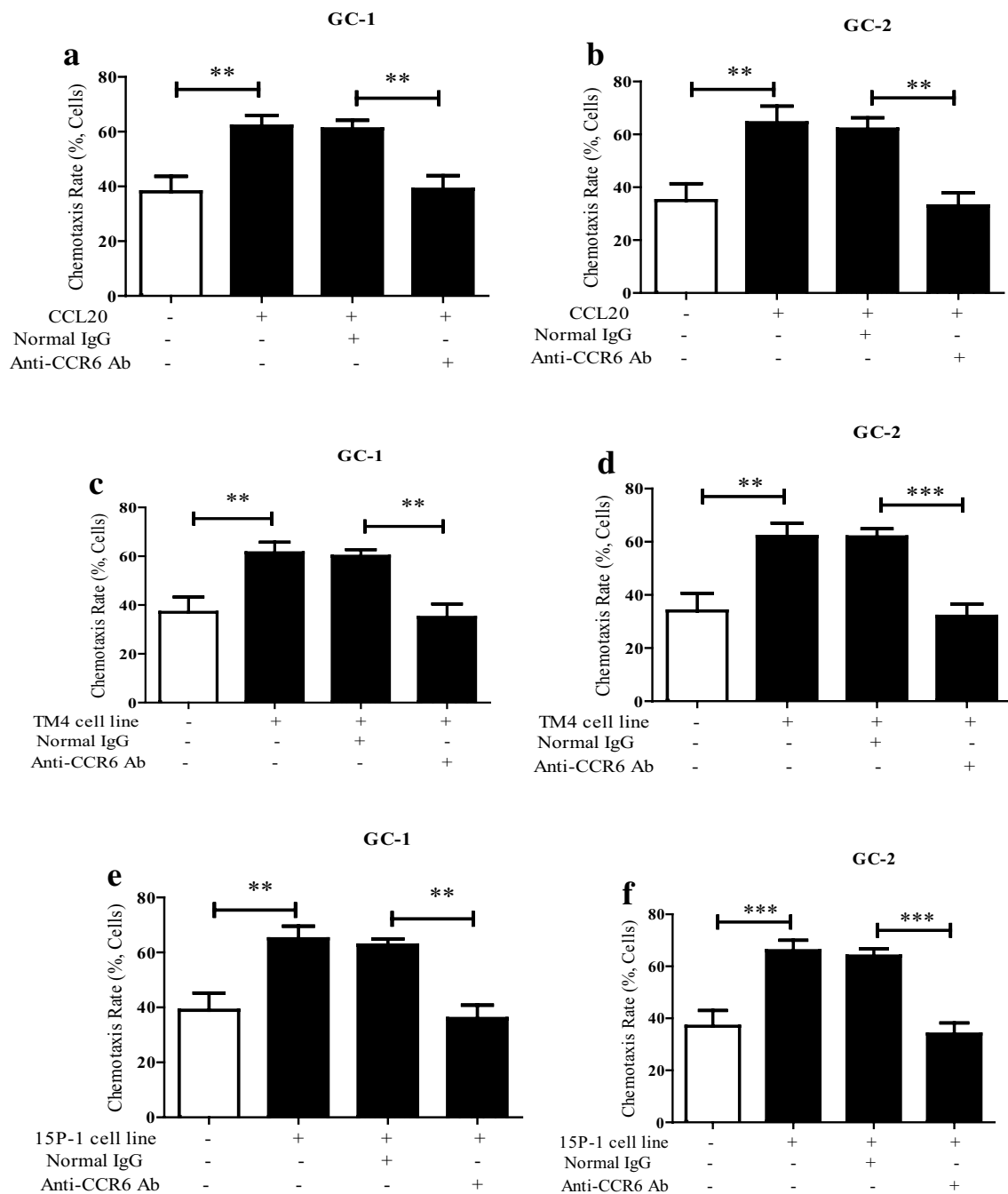


Fig. 4 The involvement of CCR6 in the chemotaxis of mouse spermatogenic cell lines (GC-1 and -2) induced by CCL20, TM4 or 15P-1 cell line in vitro. The chemotactic activity of CCL20 to GC-1 (**a**) and GC-2 (**b**), with or without the immunodepletion of anti-CCR6 antibody. The chemotactic activity of the culture supernatant of TM4 cell line to GC-1 (**c**) and GC-2 (**d**), with or without the immunodepletion of anti-CCR6 antibody. The chemotactic activity of the culture supernatant of 15P-1 cell line to GC-1 (**e**) and GC-2 (**f**), with or without the immunodepletion of anti-CCR6 antibody. Normal rabbit IgG was used as negative control. ** $p < 0.01$ and *** $p < 0.001$. $n = 10$ in each group

activated by its ligand CCL20. These findings suggest that CCR6-CCL20 interaction between spermatogenic and Sertoli cells play essential roles in spermatogenesis.

Conclusion

In conclusion, CCR6 for a receptor of CCL20 appears to be important throughout germ cell maturation, development and migration. Blockade or loss of the receptor

results in the inability of cell chemotaxis of spermatogenic cells to CCL20 or Sertoli cells. Therefore, the present findings further support an essential role of CCR6 in non-inflammatory chemotaxis, which is conducive to the inward migration of germ cells during the seminiferous epithelial cycle.

Additional files

Additional file 1: Figure S1. Expression of CCR6 in normal adult human testis. Representative western blot results showing the expression of CCR6 in normal adult human testis (n = 5). β -Tubulin was used as loading control.

Additional file 2: Figure S2. Characteristics of TNF- α localization in mouse testis. Representative immunohistochemical images showing the location of TNF- α in the testicular interstitial of mouse testis (arrow). Nuclei were counterstained with hematoxylin; At least three independent experiments were done. Scale bar = 50 μ m.

Additional file 3: Figure S3. The involvement of CCR6 in the chemotaxis of mouse spermatogenic cell lines (GC-1 and -2) induced by rDEFB1 or primary sertoli cells in vitro. The chemotactic activity of rDEFB1 to GC-1 (A) and GC-2 (B), with or without the immunodepletion of anti-CCR6 antibody. The chemotactic activity of the culture supernatant of primary Sertoli cells to GC-1 (C) and GC-2 (D), with or without the immunodepletion of anti-CCR6 antibody. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. Normal rabbit IgG was used as negative control. n = 10 in each group.

Authors' contributions

RYD and LSM designed the study, drafted the manuscript, and conducted the functional analysis. XYC, LL, LHY, and YGD collected the samples and designed and coordinated the study. ZMC and YTG provided intellectual advice. All authors read and approved the final manuscript.

Author details

¹ Reproductive Medicine Centre, Shenzhen No. 2 Hospital, Shenzhen University School of Medicine, No. 3002, Sunguang West Road, Shenzhen 518035, Guangdong, China. ² Guangdong and Shenzhen Key Laboratory of Male Reproductive Medicine and Genetics, Institute of Urology, Peking University Shenzhen Hospital, Biomedical Research Institute, Shenzhen PKU-HKUST Medical Center, Shenzhen, China.

Acknowledgements

The authors thank the patients and the family members for their cooperation during the study. The authors also thank the technicians of the Reproductive Medicine Centre of the First Affiliated Hospital of Shenzhen University for their help in semen collection and processing and for their technical assistance. The authors are grateful for the help of Miss Cailing Li with the experiments and data collection.

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

Not applicable.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Not applicable.

Funding

This work was supported by grants from the National Natural Science Foundation of China (Grant Number 81401258), Guangdong Natural Science Foundation (Grant Numbers 2014A030313652 and 2014A030313785),

Shenzhen Foundation of Science and Technology (grant number JCYJ20150330102401083), Medical Scientific Research Foundation of Guangdong (Grant Numbers A2015122 and A2015408), and Clinical Doctor—Basic Scientist Combination Foundation of Shenzhen Second People's Hospital.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Received: 14 April 2017 Accepted: 15 May 2018

Published online: 22 May 2018

References

1. Siu MK, Cheng CY. Dynamic cross-talk between cells and the extracellular matrix in the testis. *BioEssays*. 2004;26(9):978–92.
2. Boockfor FR, Schwarz LK. Effects of interleukin-6, interleukin-2, and tumor necrosis factor alpha on transferrin release from Sertoli cells in culture. *Endocrinology*. 1991;129(1):256–62.
3. Zirkin BR, et al. Testicular steroidogenesis in the aging brown Norway rat. *J Androl*. 1993;14(2):118.
4. Simon L, et al. ETV5 regulates sertoli cell chemokines involved in mouse stem/progenitor spermatogonia maintenance. *Stem Cells*. 2010;28(10):1882–92.
5. Yamazaki T, et al. CCR6 regulates the migration of inflammatory and regulatory T cells. *J Immunol*. 2008;181(12):8391–401.
6. Caballero-Campo P, et al. A role for the chemokine receptor CCR6 in mammalian sperm motility and chemotaxis. *J Cell Physiol*. 2014;229(1):68–78.
7. Patil AA, et al. Cross-species analysis of the mammalian beta-defensin gene family: presence of syntenic gene clusters and preferential expression in the male reproductive tract. *Physiol Genomics*. 2005;23(1):5–17.
8. Com E, et al. Expression of antimicrobial defensins in the male reproductive tract of rats, mice, and humans. *Biol Reprod*. 2003;68(1):95–104.
9. Diao R, et al. Deficient human beta-defensin 1 underlies male infertility associated with poor sperm motility and genital tract infection. *Sci Transl Med*. 2014;6(249):249ra108.
10. Hofmann MC, et al. Immortalized germ cells undergo meiosis in vitro. *Proc Natl Acad Sci USA*. 1994;91(12):5533–7.
11. Hofmann MC, et al. Immortalization of germ cells and somatic testicular cells using the SV40 large T antigen. *Exp Cell Res*. 1992;201(2):417–35.
12. Grandjean V, et al. Antimicrobial protection of the mouse testis: synthesis of defensins of the cryptdin family. *Biol Reprod*. 1997;57(5):1115.
13. Yang D, et al. Cutting edge: immature dendritic cells generated from monocytes in the presence of TGF-beta 1 express functional C-C chemokine receptor 6. *J Immunol*. 1999;163(4):1737–41.
14. Jaiswal MK, et al. Vacuolar-ATPase isoform a2 regulates macrophages and cytokine profile necessary for normal spermatogenesis in testis. *J Leukoc Biol*. 2014;96(2):337–47.
15. Thomas S, et al. Seminal plasma adipokine levels are correlated with functional characteristics of spermatozoa. *Fertil Steril*. 2013;99(5):1256–63.
16. Jenab S, Morris PL. Testicular leukemia inhibitory factor (LIF) and LIF receptor mediate phosphorylation of signal transducers and activators of transcription (STAT)-3 and STAT-1 and induce c-fos transcription and activator protein-1 activation in rat Sertoli but not germ cells. *Endocrinology*. 1998;139(4):1883–90.
17. Hales DB. Testicular macrophage modulation of Leydig cell steroidogenesis. *J Reprod Immunol*. 2002;57(1–2):3–18.
18. Oppermann M. Chemokine receptor CCR5: insights into structure, function, and regulation. *Cell Signal*. 2004;16(11):1201–10.
19. Sullivan R, Frenette G, Girouard J. Epididymosomes are involved in the acquisition of new sperm proteins during epididymal transit. *Asian J Androl*. 2007;9(4):483–91.
20. Elgueta R, et al. CCR6-dependent positioning of memory B cells is essential for their ability to mount a recall response to antigen. *J Immunol*. 2015;194(2):505.
21. Reimer D, et al. Early CCR6 expression on B cells modulates germinal centre kinetics and efficient antibody responses. *Immunol Cell Biol*. 2016;95(1):33–41.

22. Ohshiro K, et al. Molecular cloning of rat macrophage-stimulating protein and its involvement in the male reproductive system. *Biochem Biophys Res Commun*. 1996;227(1):273–80.
23. Gerard N, et al. Sertoli cells are the site of interleukin-1 alpha synthesis in rat testis. *Mol Cell Endocrinol*. 1991;82(1):R13–6.
24. Hakovirta H, et al. Function of interleukin-6 as an inhibitor of meiotic DNA synthesis in the rat seminiferous epithelium. *Mol Cell Endocrinol*. 1995;108(1–2):193–8.
25. Yang QE, et al. CXCL12-CXCR4 signaling is required for the maintenance of mouse spermatogonial stem cells. *J Cell Sci*. 2013;126(Pt 4):1009–20.
26. Hakovirta H, et al. MIP-1 alpha is a regulator of mitotic and meiotic DNA synthesis during spermatogenesis. *Mol Cell Endocrinol*. 1994;99(1):119–24.
27. Ghadjar P, et al. The chemokine CCL20 and its receptor CCR6 in human malignancy with focus on colorectal cancer. *Int J Cancer*. 2009;125(4):741–5.
28. Zhang XP, et al. Role of CCL20/CCR6 and the ERK signaling pathway in lung adenocarcinoma. *Oncol Lett*. 2017;14(6):8183–9.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more biomedcentral.com/submissions

