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Expression of ciliated bronchial epithelium 1 during human spermatogenesis

Christiane Pleuger, M.Sc.,[a] Daniela Fietz, Dr.Med.Vet.,[a] Katja Hartmann, Dr.Med.Vet.,[a] Hans-Christian Schuppe, Dr.Med.,[b] Wolfgang Weidner, Dr.Med.,[b] Sabine Kliesch, Dr.Med.,[c] Mark Baker, Ph.D.,[d] Moira K. O'Bryan, Ph.D.,[e] and Martin Bergmann, Dr.Rer.Medic.[a]

[a] Institute for Veterinary Anatomy, Histology und Embryology, and [b] Clinic for Urology, Pediatric Urology and Andrology, Justus Liebig University, Giessen, Germany; [c] Department of Clinical Andrology, Centre for Reproductive Medicine and Andrology, University Hospital Münster, Münster, Germany; [d] School of Environmental and Life Science, University of Newcastle, Callaghan, New South Wales, Australia; and [e] Development and Stems Cells Program, Monash Biomedicine Discovery Institute and Department of Anatomy and Developmental Biology, Monash University, Clayton, Victoria, Australia

Objective: To define the precise cellular localization of ciliated bronchial epithelium 1 (CBE1) in the human testis and test its relationship to impaired spermatogenesis.
Design: Gene expression analysis, and histologic and immunohistochemical evaluation.
Setting: University research laboratories and andrologic outpatient clinic.
Patient(s): Forty-three human testicular biopsies: 12 biopsies showing normal spermatogenesis (NSP), 8 with maturation arrest at level of spermatocytes (STA), 8 with maturation arrest at level of spermatids (SDA), 4 with scattered elongating spermatids, and 12 with Sertoli cell-only syndrome, with an additional 5 semen samples from healthy donors.
Intervention(s): None.
Main Outcome Measure(s): Evaluation of *CBE1* expression in normal as well as impaired spermatogenesis on mRNA (quantitative reverse-transcription polymerase chain reaction and in situ hybridization) and protein level (immunohistochemistry, Western blot analysis).
Result(s): In normal spermatogenesis, *CBE1* mRNA was expressed in late pachytene spermatocytes, and the protein was localized within the flagellum of elongating spermatids from stage V up to the spermiation in stage II. Immunoelectron microscopy showed CBE1 clearly associated with microtubules at the manchette, the head-tail coupling apparatus, and the flagellum, but the protein was absent in spermatozoa. Compared with normal spermatogenesis, CBE1 mRNA was statistically significantly reduced in samples with a maturation arrest at the level of round spermatids and primary spermatocytes, and was absent in samples showing Sertoli cell-only syndrome. CBE1 protein was completely missing in SDA samples showing few elongating spermatids.
Conclusion(s): Our data strongly suggest an influence of CBE1 in ciliogenesis in spermatids due to the localization at the microtubules of the elongating spermatids, indicating a role in the intramanchette and/or intraflagellar transport mechanism. The absence of CBE1 in spermatozoa suggests that CBE1 is important for the spermatid development but not for the maintenance of mature spermatozoa as a component of the flagellum. (Fertil Steril® 2017;■:■–■. ©2017 by American Society for Reproductive Medicine.)
Key Words: Intraflagellar transport, intramanchette transport, male fertility, microtubules, spermiogenesis

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Reprint requests: Christiane Pleuger, M.Sc., Institute for Veterinary Anatomy, Histology and Embryology, Justus Liebig University, Frankfurter Straße 98, 35392 Gießen, Germany (E-mail: christiane.pleuger@vetmed.uni-giessen.de).

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Microtubules play a crucial role during cell-division processes, such as mitosis and meiosis, and the development of microtubule-based structures during spermiogenesis. After meiotic reductive divisions, haploid round spermatids undergo many modifications to achieve the required characteristics for fertilization. This process includes the polarization of the nucleus with the acrosome to one side of the cell, the concomitant elongation of the

spermatid, the nuclear condensation and sperm head shaping by the manchette, as well as the assembly of the axoneme (1). The inappropriate formation of these microtubule-based structures and defects in the associated transport mechanisms along the manchette and flagellum of spermatids can lead to asthenozoospermia (reduced motility) and/or teratozoospermia (abnormal morphology), thus causing male infertility (2).

The formation of the axoneme of the sperm flagellum shows parallels to the development of motile cilia in other tissues, such as the brain and the lung (3). The axoneme, the core of the flagellum, consists of a centrally located microtubule pair surrounded by nine outer microtubule doublets that are connected by the dynein heavy chains of the inner and outer dynein arms. Axoneme formation in many cilia and likely in the sperm tail involves a microtubule-based protein system known as intraflagellar transport (IFT). The mammalian sperm tail has additional characteristic peri-axonemal structures, such as nine outer dense fibers, the mitochondrial sheath, and fibrous sheath and is connected to the nucleus via the connecting piece (4). The structural features enable motility of the mature sperm and maintain structural integrity (5).

Concordant with the onset of spermatid head elongation, the transient microtubule-based manchette comes into existence and persists until the elongation and condensation of the spermatid nucleus is completed (6). The manchette is thought to serve essential roles in nuclear shaping and to act as a protein transport platform for proteins involved in tail formation, known as intramanchette transport (IMT) (7).

While not completely characterized and temporally distinct, the IFT likely shares similar molecular components to IMT. It is proposed that in both transport mechanisms, a multicomplex protein raft linked to cargo proteins and vesicles moves along a microtubule track (6, 8, 9). During IFT, essential proteins for the assembly and maintenance of the flagellum are transported in an anterograde direction by kinesins to the distal part of the flagellum (10) and in a retrograde direction by dyneins back toward the basal body (11). It is proposed that the IMT, which is transient just like the manchette itself, additionally enables an exchange of cargo between the Golgi, nucleus, and cytoplasm as well as the head-tail coupling apparatus (HTCA) (2, 8, 9, 12).

Ciliated bronchial epithelium 1 (CBE1 alias SMRP1, 1110017D15Rik, C9orf24, NYD-SP22) is a protein that has been described in association with ciliated cells in bronchial tissue (13) and in the manchette of murine elongating spermatids (14). During pulmonary development, CBE1 shows a biphasic expression. CBE1 expression starts during the formation of the lung buds and shows a further expression in later stages (pseudoglandular stage) concurrent with the expression of the transcription factor forkhead box factor FOXJ1 (hepatocyte nuclear factor-3/forkhead homologue 4) (15), which is closely linked to ciliogenesis (16). Although these data indicate a role for CBE1 in establishing differentiation of the mucociliary epithelium (15), CBE1 is localized intracellularly in ciliated epithelial cells but not in the cilia structure (13).

Previous investigations of the murine orthologue (called *Smrp1* spermatid-manchette-related protein 1 in the original study) detected the protein at the spermatid manchette around the nucleus where it colocalized with α-tubulin. Although three mRNA variants have been described, only one transcript variant was translated into the protein (14). To date, no precise molecular function has been described for CBE1. Although the murine orthologue shows a clear localization at the spermatid manchette (14), it is unknown whether CBE1 is either an integral part of this structure or it plays a role in the cargo transport. To clarify its potential relevance to human spermatogenesis, we investigated the mRNA expression and protein localization of CBE1 in human testes with normal and impaired spermatogenesis to gain information regarding the molecular function and a possible association with the phenotype of maturation arrests.

MATERIAL AND METHODS

Testicular Tissue and Semen Samples

Human testicular biopsy samples were obtained (with consent) from patients of the Centre of Reproductive Medicine and Andrology of the University Hospital in Münster (Germany) and the Department of Urology and Andrology of the University Hospital in Giessen (Germany). The surgical procedure was indicated because of obstructive (refertilization after vasectomy) or nonobstructive azoospermia as defined using the criteria according to Bergmann and Kliesch (17).

In total, 75 testicular biopsy samples were used (44 samples to analyze mRNA expression and 31 to evaluate the protein localization in normal and impaired spermatogenesis). Immediately after surgical removal, the testicular tissues were immersed in Bouin's solution and ultimately embedded in paraffin. We stained 5-μm-thick sections with hematoxylin and eosin (H&E) to evaluate the quality of the spermatogenesis according to the score count protocol of Bergmann and Kliesch (17). The stages of spermatogenesis were differentiated in accordance with Clermont (18). To analyze the mRNA expression, we selected 12 biopsy samples showing normal spermatogenesis (NSP); 8 biopsy samples with a maturation arrest at the level of early round spermatids (SDA), whereby samples showed scattered elongating spermatids; 12 biopsy samples with a maturation arrest at the level of primary spermatocytes (STA); and 12 biopsy samples showing Sertoli cell-only syndrome (SCO).

Human ejaculates (n = 5) were received from five different healthy volunteers. A semen analysis was performed according to the World Health Organization's 2010 guidelines to measure sperm concentration, motility, morphology, and vitality (19). After the evaluation, the ejaculates were centrifuged at 500 $\times$ *g* for 15 minutes, and the pellet was washed three times with sperm washing medium containing 5 mg/mL of human serum albumin (Irvine Scientific) and stored at -20°C.

The study was performed following the declaration of Helsinki. Written informed consent was obtained from all men involved. The study was approved by the ethics committee of the medical faculty of the Justus-Liebig-University Giessen (AZ 100/07; AZ 32/11).