

BRIEF REPORT

Sperm Protein 17 Is Expressed in Human Somatic Ciliated Epithelia

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SUMMARY It was once believed that sperm protein 17 (Sp17) was expressed exclusively in the testis and that its sole function was to bind to the oocyte during fertilization. However, immunohistochemistry of the human respiratory airways and reproductive systems show that it is abundant in ciliated cells but not in human cells with stereocilia and microvilli. The high degree of sequence conservation throughout its N-terminal half, and the presence of an A-kinase anchoring protein (AKAP)-binding motif within this region, suggest that Sp17 plays a regulatory role in a PKA-independent AKAP complex in both male germinal and ciliated somatic cells. (*J Histochem Cytochem* 52:549–554, 2004)

KEY WORDS

sperm protein 17
respiratory system
reproductive system
cilia
microvilli
immunohistochemistry

Sp17 is a highly conserved mammalian protein that was originally included as a member of the rabbit sperm auto-antigen (RSA) family (O’Rand et al. 1988). Northern blotting analyses of mouse tissues revealed that Sp17 was not present in any of the mouse somatic tissues examined (brain, heart, kidney, liver, lung, skeletal muscle, and spleen) but was specific to the testis. However, mRNA for Sp17 has been detected by RT-PCR in a range of murine somatic tissues (albeit in much lower amounts than those found in testis) and in a panel of cDNAs obtained from different human somatic tissues, including brain, heart, kidney, liver, lung, pancreas, placenta, and skeletal muscle (Frayne and Hall 2002).

The expression pattern of this antigen in disease-free samples of human testis and ejaculated spermatozoa

was first investigated using self-produced mouse anti-human Sp17 antibodies (Grizzi et al. 2003). Interestingly, the flagella of the ejaculated spermatozoa and the spermatozoa identified in the lumen of the seminiferous tubules were immunopositive for Sp17. Because flagella are structurally very similar to cilia (except that they are usually very much longer), the aim of the present immunohistochemical (IHC) study was to investigate whether Sp17 is also expressed in human somatic ciliated cells. The study was then extended to other cell apical specializations, i.e., stereocilia and microvilli.

The formalin-fixed, paraffin-embedded specimens with normal histological features included three samples each of lung, trachea, and larynx, representing the respiratory airways, and the fallopian tube and ductuli efferentes for the female and male reproductive systems, respectively. Nine histologically normal samples of the epididymis, ductus deferenti, and kidney were used to evaluate the expression of Sp17 in cells with stereocilia, and three samples of the duodenum to recognize its expression in cells with microvilli. Cytological samples of ejaculated spermatozoa collected from three healthy, fertile donors were used as a positive staining control.

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Received for publication October 6, 2003; accepted December 17, 2003 (3B6185).

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The fallopian tube and lung homogenates were Western blotted with self-produced mouse anti-human Sp17 antibodies (Grizzi et al. 2003) to demonstrate the specificity of the antibodies in the labeled histological sections. The homogenates were clarified by centrifugation ($2000 \times g$, 10 min), electrophoresed under reducing and denaturing conditions on a 12% polyacrylamide gel, and transferred to nitrocellulose membranes. The membranes were incubated overnight at 4C with monoclonal mouse anti-Sp17, and the immunoreactive bands were revealed by the Opti-4CN substrate (Amplified Opti-4CN; BioRad, Milan, Italy).

The specimens were fixed in 10% neutral buffered formalin and embedded in paraffin, and 2- μ m hematoxylin-eosin-stained sections were histologically analyzed under a light microscope (Leica DMLA; Milan, Italy).

Additional 2- μ m sections were cut and processed for IHC. After deparaffining and rehydration, the antigen was retrieved by immersing the sections in a bath (DAKO; Milan, Italy) for 45 min at 98C in a freshly made EDTA 1 mM solution, incubating them with 3% H_2O_2 for 15 min to quench endogenous peroxidase activity, and then treating them with primary antibodies at room temperature (RT) for 2 hr, or 1 mg/ml of mouse IgG1 (DAKO) as a negative control. The sections were then incubated for 30 min using the DAKO Envision system. 3,3'-Diaminobenzidine tetrahydrochloride (Sigma; St Louis, MO) was used as a chromogen to yield brown reaction products. Immunocytochemistry was also performed on cytological samples of ejaculated spermatozoa as previously described (Grizzi et al. 2003).

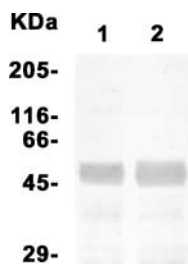


Figure 1 Western blots of fallopian tube (Lane 1) and lung homogenates (Lane 2) showing the immunoreactive bands revealed by affinity-purified mouse anti-rHSp17 (dilution 1:1000). The reference molecular weights are indicated at left. According to Lea et al. (1996), Adoyo et al. (1997), and Grizzi et al. (2003), the strongly detected 54-kD band is consistent with the multimeric form of Sp17. An additional band at 24.5 kD was also faintly detectable in both samples, as previously described.

Figure 1 shows Western blots of fallopian tube and lung homogenates, which demonstrate the specificity of the antibody on the labeled histological sections. A strong immunoreactive band of 54 kD was detected in both samples. An additional band at 24.5 kD was also faintly detectable in both samples, as previously described (Grizzi et al. 2003).

At higher magnification (Figure 2), Sp17 was clearly detectable throughout the principal part of the flagellum, thus confirming previous results (Grizzi et al. 2003).

Sp17 was highly expressed in the ductuli efferentes, being localized in the lining cells and throughout the length of their cilia (Figure 3A). The non-ciliated columnar and basal cells were always immunonegative for Sp17. Sp17 was also recognized in all fallopian tube samples (Figure 3B), in which the motile cilia were highly positive and moderate amounts of protein were detectable in the cell cytoplasm, but it was not expressed in the non-ciliated cells lining the oviduct epithelium, i.e., secretory and basal cells (Figure 3B).

All ciliated epithelial cells of the respiratory system were immunopositive for Sp17, particularly the ciliated columnar cells lining the larynx epithelium (Figure 3C) and in the tracheal epithelium (Figure 3D). Brush cells and the deeper layers were immunonegative. The ciliated cells of the intrapulmonary bronchi (Figure 3E) were immunopositive, with staining being detected up to the level of the cartilage-free lobular bronchiole (Figure 3F). No staining was observed where each bronchiole divides into two or more respiratory bronchioles with scattered alveoli.

No protein Sp17 was expressed in the cells of the kidney (Figure 4A), epididymis (Figure 4B), or ductus deferenti (Figure 4C), all of which are examples of ep-

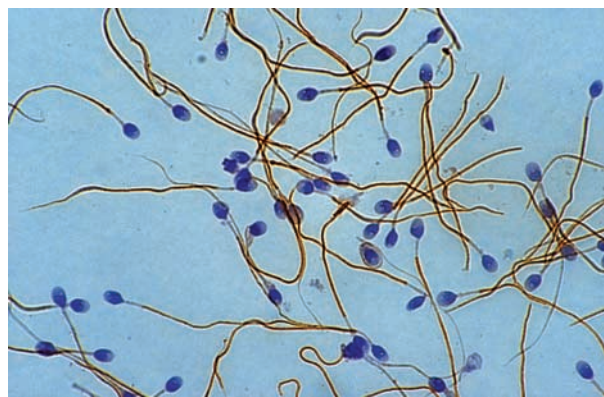


Figure 2 Sp17 has two distinct populations, one immunopositive and the other immunonegative. Sp17 is clearly detectable throughout the principal part of the flagellum, but the intermediate piece, head, and acrosome vesicle are immunonegative. Magnification $\times 100$. The IgG controls were always negative (not shown).

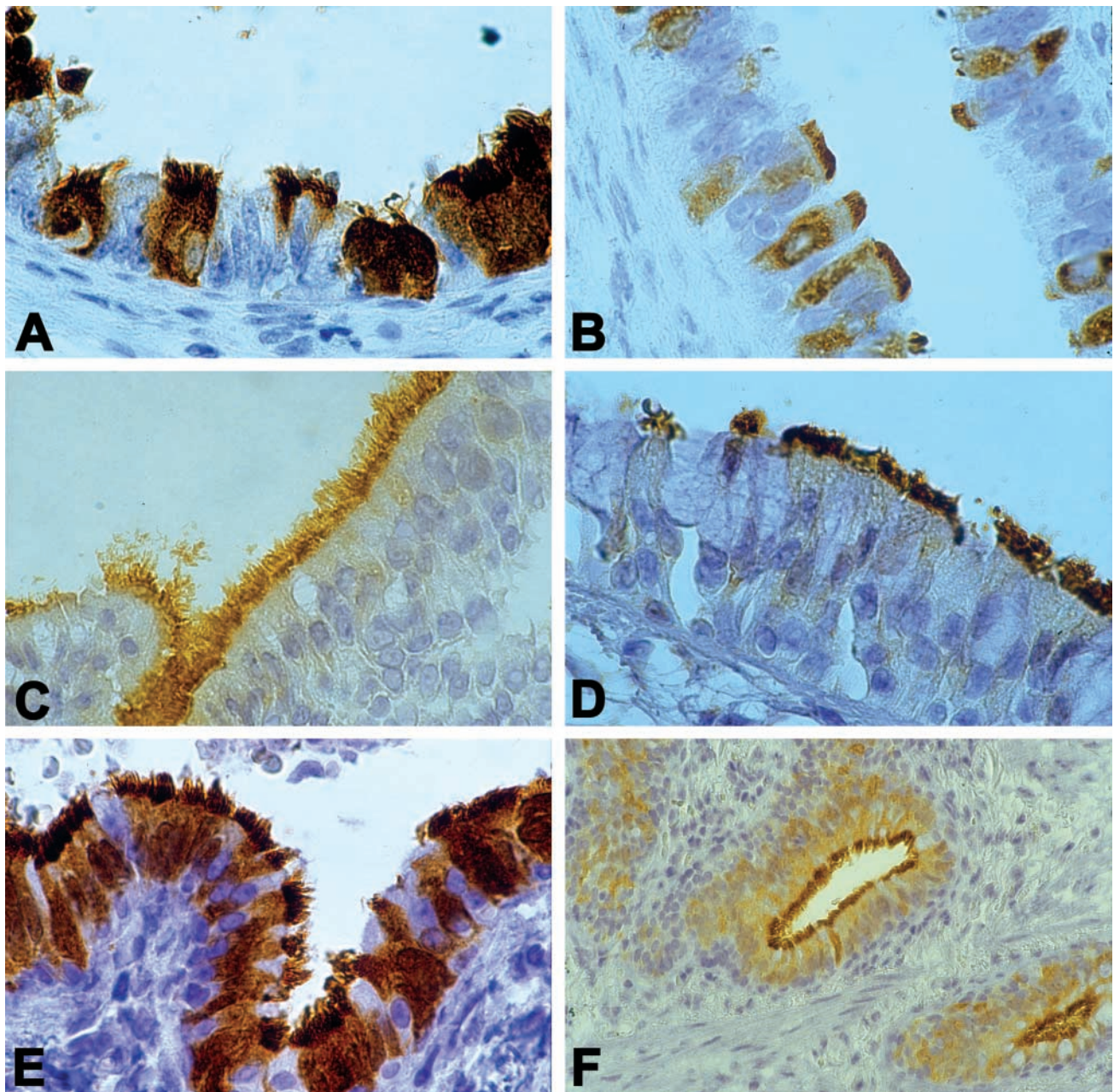


Figure 3 Detection of Sp17 in somatic ciliated epithelia. Histological sections of ductuli efferentes in the male germinal tract (A), fallopian tubes of the female reproductive system (B), and in larynx (C), trachea (D), and lung (E). Staining was detected up to the level of the cartilage-free lobular bronchioles (F). Sp17 is expressed in the cytoplasm of lining epithelial cells and their cilia. IgG controls were always negative (not shown). Magnifications: A–E $\times 100$; F $\times 40$.

ithelia with stereocilia in the apical region. The microvillous epithelium of the duodenum was also immunonegative.

Cilia and flagella appeared very early in evolution as a means of allowing unicellular organisms to move in water. The high degree of sequence conservation

between the flagellar proteins of unicellular organisms and mammalian ciliary proteins suggests that the functional role of the genes encoding cilia has been preserved but that the functions of cilia in various human primate processes (such as left–right axis pattern formation, cerebrospinal fluid flow, sensory reception,

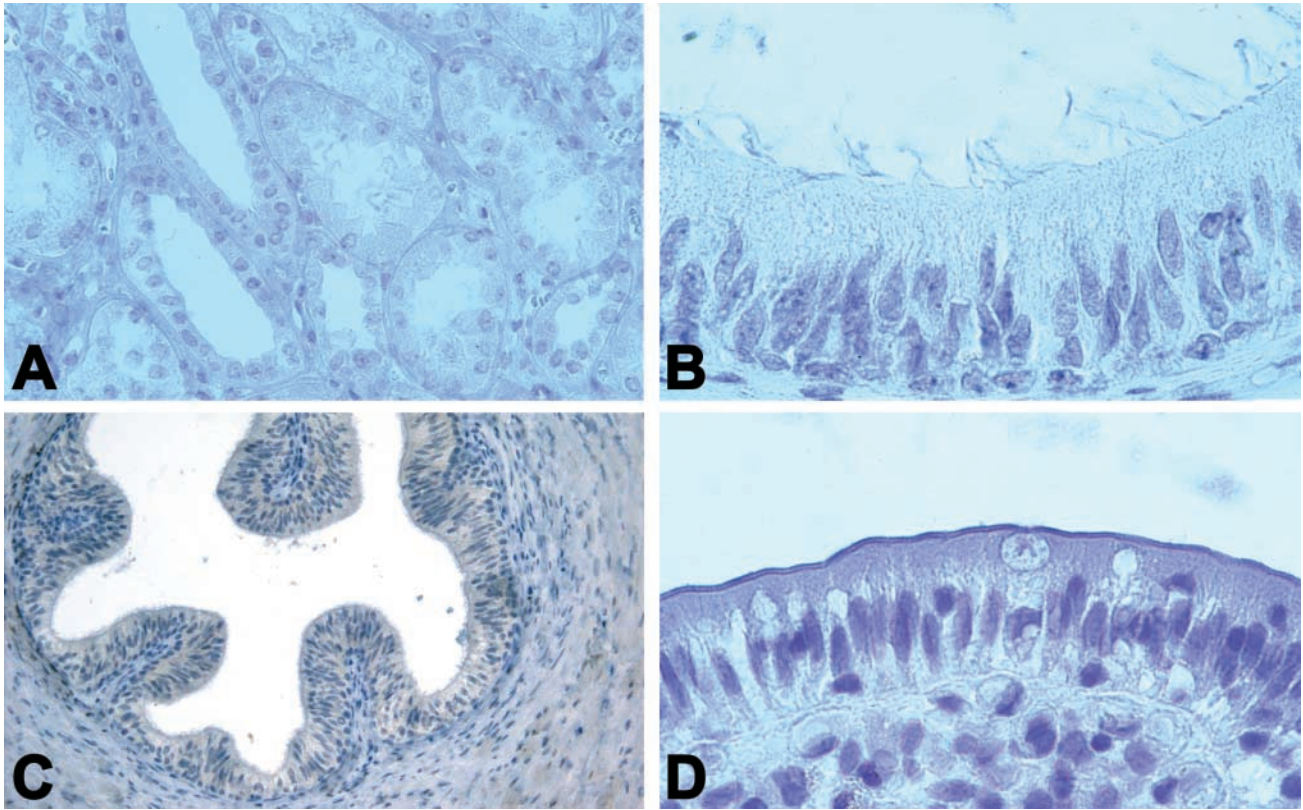


Figure 4 No protein Sp17 expression was found in the stereociliated cells of the kidney (A), epididymis (B), or ductus deferenti (C). The microvillous duodenal epithelium was also immunonegative (D). IgG controls were always negative (not shown). Magnifications: A $\times 40$; B,D $\times 100$; C $\times 20$.

mucociliary clearance, and renal physiology) indicate that they have become versatile biological tools (Ibanez-Tallon et al. 2003).

An important step towards a complete understanding of ciliary growth and function is to identify the proteins that make up their skeleton (axoneme). In an attempt to identify all of the components of human respiratory cilia, Ostrowski et al. (2002) recently made a comprehensive proteomic analysis of isolated human ciliary axonemes. The use of 2D PAGE led to a highly reproducible 2D map of more than 240 well-resolved components, a number of which had sequences matching those of proteins previously established as being sperm- or testis-specific. The peptides included Sp17, a mannose-binding protein originally included in the family of low molecular weight rabbit sperm autoantigens.

Using self-produced mouse anti-human Sp17 antibodies, we first investigated the expression pattern of this antigen in disease-free samples of human testis and ejaculated spermatozoa (Grizzi et al. 2003). Interestingly, the flagella of the ejaculated spermatozoa

and the spermatozoa identified in the lumen of the seminiferous tubules were both immunopositive for Sp17. Because the flagella of spermatozoa are structurally very similar to cilia and it has been widely suggested that they may have a eukaryotic origin, we investigated whether Sp17 is expressed in human somatic ciliated cells and then extended the study to cells with stereocilia or microvilli.

We found that Sp17 is synthesized in the ciliated epithelia of the human respiratory airways and the male and female reproductive systems (Figures 4A–4C) but not in cells with stereocilia or microvilli (Figure 4D), which allows the following conclusions to be drawn. (a) The present data extend recent studies in which a proteomic analysis of human respiratory cilia revealed the expression of Sp17 and an RT-PCR analysis showed Sp17 transcripts in human lung insofar as we found protein Sp17 not only in the ciliated cells of the respiratory system but also in those of the male and female reproductive systems. (b) Like immature germ cells (spermatogonia), the basal cells of the respiratory and reproductive ciliated epithelia were immu-

nonegative for Sp17, suggesting that Sp17 synthesis begins at a specific stage of cell differentiation. (c) Sp17 was originally cloned and sequenced in the rabbit as a specific auto-antigen that binds sulfate carbohydrates and the zona pellucida of the oocyte, and it has been shown that RSA antiserum inhibits fertilization in vivo and in vitro. Sp17 has been attributed with the ability to bind complex carbohydrates in vitro because it contains two conserved heparin-binding motifs (Wen et al. 2001). Although in vitro fertilization is inhibited by antiserum against the RSA family of rabbit sperm proteins, the family also includes antigens other than Sp17. Together with the present data, the lack of any direct evidence that Sp17-specific antiserum alone inhibits sperm-zona pellucida interactions (Frayne and Hall 2002) suggests that the proposed role of Sp17 in zona pellucida binding is unlikely to be its principal function, and Sp17 immunolocalization throughout the principal part of the sperm flagellum and in somatic cilia implies alternative or additional roles. In a recent re-evaluation of Sp17, Frayne and Hall (2002) noted that its highly conserved N-terminal domain contains a motif (the first 74 amino acids) that is very similar to the N-terminal sequence of the regulatory subunit II of cAMP-dependent protein kinase A (PKA RII), which is essential for protein dimerization and interactions with A-kinase anchoring proteins (AKAPs). Although an AKAP-binding function has not yet been demonstrated, it is plausible that Sp17 plays a regulatory role in a PKA-independent AKAP complex in both ciliated somatic and germinal cells. AKAPs represent a family of sequence-unrelated proteins classified exclusively by their ability to bind PKA in vitro, which possess targeting domains that mediate their attachment to the cytoskeleton, plasma membrane, or intracellular organelles (Colledge and Scott 1999). Three sperm-specific AKAP-binding proteins have thus far been identified (ropporin, AKAP-associated sperm protein and fibrousheathin II), all of which are localized in the fibrous sheath of the sperm tail. Likewise, Sp17 has been found in sperm flagellum by immunocytochemistry and in the fibrous sheath by electron microscopy. Interestingly, Kultgen et al. (2002) have recently provided the first biochemical data showing that a PKA pool is localized in human ciliary axonemes. They also identified the first human A-kinase anchoring protein targeted to the ciliary axoneme (called AKAP28 in the cilia of the columnar cells of the respiratory airways but not in goblet and basal cells) and suggested that it localizes PKA to a position in the axoneme where it can readily interact with its substrate. This specific compartmentalization probably plays a role in the regulation of outer dynein arm activity and the control of ciliary beat frequency. Similar results have been

obtained in hamster oviducts, in which Morales et al. (2000) found that adenosine (a pharmacological agent) stimulated ciliary beat frequency in an adenylyl cyclase-dependent manner. All of these data support the hypothesis that Sp17 plays an important signaling role in both somatic and germinal cells. (d) The aberrant expression of Sp17 in cancer tissues has suggested that it may be useful as a key target for immunotherapy in multiple myeloma and ovarian cancer (Chiriva-Internati et al. 2002a,b) although, as previously discussed (Chiriva-Internati et al. 2003), the present results confirm the need for new laboratory tests to establish its safety and applicability in specific immunotherapeutic procedures.

In conclusion, the extremely high sequence conservation in the N-terminal half of Sp17 implies that it is functionally very important. The fact that this is not the region that may be involved with sperm-egg interactions, the clear sequence homology within this region to the binding site of a range of AKAP ligands, and the presence of Sp17 in somatic ciliated cells strongly support the idea that zona pellucida binding is not the primary function of Sp17. These exciting new findings should stimulate further electron microscopic and biochemical experimental studies.

Acknowledgments

Supported by the Women's Health Research Institute, Texas Tech University, Amarillo, and the Michele Rodriguez Foundation, Istituto Scientifico per le Misure Quantitative in Medicina, Milan, Italy.

We are very grateful to Dr Marco Alloisio and Mr Kevin Smart for their valuable support.

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