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LETTER TO THE EDITOR

Sperm Biology

Immunodetection of Tau microtubule-associated protein in human sperm and testis

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Dear Editor,

The cytosolic protein Tau is naturally present in human neurons, where it has a pivotal role in controlling microtubule stability.¹ Hyperphosphorylation of Tau (observed during neurodegenerative diseases, such as Alzheimer's disease) impairs the protein's ability to bind microtubules. This results in microtubule disassembly and the formation of Tau aggregates.^{2,3} Tau protein is also widely expressed in peripheral tissues.⁴ In the male reproductive system, screening for Tau has focused solely on the rodent and bovine testis.^{4,5} In the present study, we used immunofluorescence and immunoenzymatic techniques (with a Tau-specific antibody)⁶ to investigate the presence of Tau protein in human ejaculated sperm and testicular tissue. We studied (i) five semen samples from normozoospermic men and (ii) testicular biopsies taken from two men suffering from obstructive azoospermia (in whom spermatogenesis was normal). The men were recruited by our university hospital's andrology clinic, and all provided their written, informed consent to participation. Briefly, washed, smeared sperm samples were incubated with normal goat serum and then with rabbit anti-C-terminus Tau antibody raised against synthetic peptides encoding the last 15 amino acids of Tau protein (dilution: 1:1000). The slides were revealed with Alexa 488-conjugated goat anti-rabbit antibodies (Molecular Probes®, Invitrogen SARL, Cergy Pontoise, France). Dewaxed 5 µm paraffin sections were microwaved and incubated with normal sheep or goat serum and then with rabbit anti-C-terminus Tau antibody (dilution: 1:1000). In immunoenzymatic experiments, the sections were revealed with a biotinylated sheep anti-rabbit immunoglobulin G (dilution: 1:150) and then an avidin/biotinylated horseradish peroxidase complex (both from Vector Laboratories, AbCys, Paris, France). In immunofluorescence experiments, the sections were revealed with fluorescent goat anti-rabbit Alexa 568 (Molecular Probes®, Invitrogen SARL, Cergy Pontoise, France). After further washing, the slides were coverslipped using Vectashield mounting medium for fluorescence with DAPI (Vector Laboratories, AbCys). In control experiments, the

primary antibody was omitted. The slides were observed, and images were acquired with a confocal LSM710 microscope and an Axioplan microscope (both from Zeiss, Göttingen, Germany).

Tau immunostaining was detected in all smeared spermatozoa (**Figure 1a**). The Tau protein was located in the sperm midpiece. In testicular tissue, Tau immunoreactivity was observed in both spermatocytes and spermatids (**Figure 1b**). In spermatocytes, cytoplasmic fluorescent dots were located all around the nucleus (**Figure 1c**). In elongated spermatids, the immunolabeled zone was situated behind the nucleus (**Figure 1d**). No staining was observed in spermatozoa or in tissue sections of control samples lacking primary antibody (**Figure 1e and 1f**).

Over the last decades, few researchers have studied the presence of Tau protein in the reproductive tract. Two eminent, early studies reported the presence of Tau protein in the rodent and bovine testis.^{4,5} In human germ cells and spermatozoa, Tau may be involved in the regulation of microtubule proteins. The spermatozoon's main microtubule-organizing centre is the centrosome complex. It has a role in sperm and zygote aster formation, and the development of the bipolar mitotic apparatus.⁷ Dysfunction of the centrosome complex will result in developmental abnormalities after fertilization. The epithelium of seminiferous tubules has a vast network of microtubules (particularly within the manchette, the axoneme, and the mitotic and meiotic spindles of germ cells).⁸ The presence of Tau in human testicular tissue suggests that this protein has a role in meiotic division in male germ cells and during their extension to spermatozoa. As has been suggested in the context of tauopathies,⁹ the aggregation, hyperphosphorylation and/or ubiquitination of Tau might impair sperm microtubule function and transport.

In summary, this study reports the immunoeexpression of Tau microtubule-associated protein in human sperm and testis. We hypothesize that Tau is involved in the development of sperm in a man and thus may represent an additional parameter for sperm function in male fertility.

AUTHOR CONTRIBUTIONS

JS carried out the immunohistochemistry and wrote the manuscript; FJ and CB participated in the design of the study; MLCB and NS revised the manuscript critically; JMR and FM participated in the retrieve of the testicular samples; MT performed the confocal microscopy analysis; LB and VM supervised the experiments. All authors read and approved the final manuscript.

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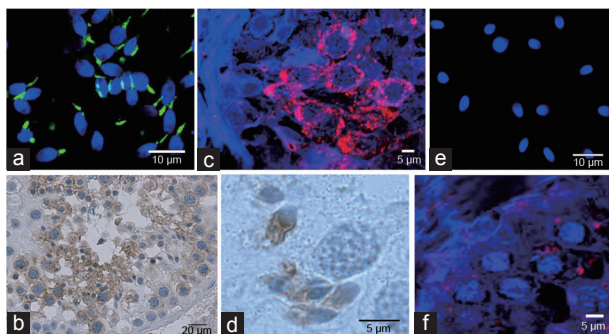


Figure 1: Micrographs of human ejaculated sperm and testicular tissue treated with anti-Tau antibody (**a-d**) and controls (**e** and **f**). (**a**) In spermatozoa, fluorescent Tau labelling is observed in the midpiece. (**b**) In testicular tissue, Tau immunoreactivity is observed in both spermatocytes and spermatids. (**c**) In spermatocytes, cytoplasmic fluorescent dots are located all around the nucleus. (**d**) In elongated spermatids, the immunolabelled zone is located behind the nucleus.

COMPETING INTERESTS

The authors declare no competing interests.

REFERENCES

- 1 Cárdenas AM, Ardiles AO, Barraza N, Baéz-Matus X, Caviades P. Role of tau protein in neuronal damage in Alzheimer's disease and Down syndrome. *Arch Med Res*

2012; 43: 645–54.

- 2 Billingsley ML, Kincaid RL. Regulated phosphorylation and dephosphorylation of tau protein: effects on microtubule interaction, intracellular trafficking and neurodegeneration. *Biochem J* 1997; 323: 577–91.
- 3 Alonso A, Zaidi T, Novak M, Grundke-Iqbal I, Iqbal K. Hyperphosphorylation induces self-assembly of tau into tangles of paired helical filaments/straight filaments. *Proc Natl Acad Sci USA* 2001; 98: 6923–8.
- 4 Gu Y, Oyama F, Ihara Y. Tau is widely expressed in rat tissues. *J Neurochem* 1996; 67: 1235–44.
- 5 Ashman JB, Hall ES, Eveleth J, Boekelheide K. Tau, the neuronal heat-stable microtubule-associated protein, is also present in the cross-linked microtubule network of the testicular spermatid manchette. *Biol Reprod* 1992; 46: 120–9.
- 6 Sergeant N, Sablonnière B, Schraen-Maschke S, Ghestem A, Maurage CA, *et al.* Dysregulation of human brain microtubule-associated tau mRNA maturation in myotonic dystrophy type 1. *Hum Mol Genet* 2001; 10: 2143–55.
- 7 Schatten H, Sun QY. The functional significance of centrosomes in mammalian meiosis, fertilization, development, nuclear transfer, and stem cell differentiation. *Environ Mol Mutagen* 2009; 50: 620–36.
- 8 Kierszenbaum AL, Tres LL. The acrosome-acroplaxome-manchette complex and the shaping of the spermatid head. *Arch Histol Cytol* 2004; 67: 271–84.
- 9 Mandelkow EM, Mandelkow E. Biochemistry and cell biology of tau protein in neurofibrillary degeneration. *Cold Spring Harb Perspect Med* 2012; 2: a006247.

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