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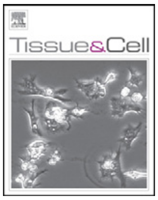

Primary cilia in the basal cells of equine epididymis: A serendipitous finding

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ABSTRACT

Occurrence of a solitary cilium was an unexpected discovery while studying the ultrastructure of epididymal epithelium in equidae. Primary cilia were detected in epididymal basal cells of all individuals of the equines studied – horses, donkey and mules – independently from age and tract of the duct, emerging from the basal cell surface and insinuating into the intercellular spaces. More rarely solitary cilia occurred also at the luminal surface of the principal cells. The ciliary apparatus was constituted by a structurally typical basal body continuous with the finger-like ciliary shaft extending from the cell surface, and an adjacent centriole oriented at right angles to the basal body. The cilium was structured as the typical primary, non-motile cilia found in many mammalian cells, having a 9 + 0 microtubular pattern. The basal diplosome was randomly associated with other cellular organelles including the Golgi complex, the endoplasmic reticulum, the microfilament network, the plasma membrane, vesicles and pits.

Primary ciliogenesis is a new and unexpected finding in the epididymal epithelium. A monitoring role of luminal factors and extracellular liquids might be attributed to this organelle, likely acting as chemical receptor of the luminal environment, thus modulating the epithelial function by a cell-to-cell crosstalk involving the entire epithelium.

1. Introduction

More than thirty-five years ago David W. Hamilton, expert on epididymal structure and function, had to affirm that “epididymal basal cells are an enigma” (Hamilton, 1975). At that time, basal cells were attributed a stem function in the epithelium renewal (Hamilton, 1975; Ramos and Dym, 1977). Soon later, the opposite view, that principal cells might give rise to basal cells, was proposed by Sun and Flickinger (1982), a hypothesis not compatible with the low mitotic activity in the epididymis (Sujarit and Jones, 1991). A simple mechanical role in giving stability to the epithelium was also suggested, owing to the presence of filament bundles and membrane infoldings (Abe and Takano, 1989). A scavenger role was later proposed for equine epididymal basal cells, acting as macrophages having the task to remove from the epithelium waste matter derived from the endocytotic and spermatophagic activity of the principal cells (Arrighi et al., 1991, 1993, 1994). In the same years, macrophage-like qualities of basal cells were hypothesized (Yeung et al., 1994; Seiler et al., 1998) together with their possible extratubular origin from circulating monocytes (Holschbach and Cooper, 2002).

The most recent interpretation for the role of epididymal basal cells is that they might scan and sense the luminal environment of pseudostratified epithelia and modulate epithelial function by a mechanism involving crosstalk with other epithelial cells (Shum et al., 2008). This might be a paradigm shared by all basal cells residing in the so-called pseudostratified epithelia.

The presence of a primary cilium emerging from the basal cell surface and insinuating into intercellular spaces was accidently found during ultrastructural studies on the epididymis in equidae. Although serendipitous in nature, the discovery of primary cilia in epididymal basal cells is a convincing finding, which completes the information on the likely functional roles most recently attributed to this enigmatic cell type.

2. Materials and methods

Specimens were collected at slaughtering or at castration from healthy pubertal horses ($n = 3$), donkeys ($n = 3$) and mules ($n = 3$) aged from 4 to 15 years. Samples were taken from the different epididymis regions, which are macroscopically recognizable: two successive samples were collected at the *caput* level, three at the *corpus* level and one in the *cauda* (Nicander, 1958).

Pieces intended for histological examination were fixed in formalin 10% for 24 h at 4 °C. After fixation, fragments were dehydrated in a graded series of ethanol and embedded in paraffin. Serial sections were cut at 4 μm thickness, de-waxed, and stained with routine hematoxylin and eosin (H&E).

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Pieces intended for electron microscopy were trimmed and fixed in 2.5% glutaraldeyde in 0.1 M phosphate buffer at pH 7.4 for 3 h, then post-fixed in 1% OsO_4 in the same buffer for 2 h. Fragments were then dehydrated through a graded series of ethanol and embedded in EPON 812.

Ultrathin sections were obtained by an ultramicrotome (Ultratome IV, LKB, Sweden) collected on uncoated copper grids and counterstained with uranyl acetate and lead citrate, then examined and photographed under a Zeiss EM 109.

3. Results

Epididymal epithelium in equidae was composed of principal cells, apical cells and basal cell. As in many other mammals, principal and apical cells were columnar whereas basal cells were roundish and rested on the basal lamina without reaching the lumen. Migrating elements such as lymphocytes and macrophages were also seen. LM pictures from the *corpus* (Fig. 1a) and *cauda* (Fig. 1b) of the horse epididymis are shown to orientate the reader. Basal cells were apparently more numerous and large in the corpus region.

Ultrastructure of the epithelium lining the epididymis in the horse, donkey and mule has been previously described in detail (Romanello et al., 1985; Arrighi et al., 1991, 1993). A low magnification EM picture depicting the most basal region of the mule epididymal epithelium is shown in Fig. 1c. Morphology of the equine epididymal basal cell is typically characterized by the roundish shape, large, round and euchromatic nucleus and a cytoplasm poor of organelles (Fig. 1d). The cellular profile is characterized by cytoplasmic extensions and membrane digitations by which the basal cell connect to adjacent epithelial cells. The usual junctional devices were present, joining the basal cells to adjacent epithelial cells all along lateral cell surface. Between the above-mentioned junctions, the facing cell membranes were separated from each other in such a way that a tiny space was often present between them (Figs. 1d and 2). The cell region facing the basal membrane was prevalently straight, connected to the basal lamina by hemidesmosomes (Fig. 2c). Frequently, micropinocytotic vesicles were present.

In the cytoplasm, microfilament bundles were often prominent especially in the area surrounding the nucleus (Figs. 1 and 2c, d). Scattered profiles of endoplasmic reticulum, few mitochondria and a poorly developed Golgi apparatus were also present, without a precise localization within the cytoplasm. Residual bodies having a heterogeneous aspect were often present (Fig. 2a), together with lipid globules.

The presence of solitary cilia was observed in the epididymal basal cells of all individuals studied, independently of species, age and epididymal tract considered (Figs. 1d and 2). Solitary cilia were more frequently observed in the basal cells of the *corpus* region. They originated from the basal cell surface, frequently extending in a tunnel-like invagination of the cell membrane and thereafter insinuating into the intercellular space (Fig. 2a, b and d). Rarely was the cilium followed for a relatively long distance among adjacent cells (Fig. 2a) whereas more often it disappeared from the plane of section after emerging straight from the cellular surface (Fig. 2b, d and e). The place where the cilium emerged was not fixed, and it never appeared facing the basement membrane.

The ciliary apparatus was randomly associated with other cellular organelles including the Golgi complex, the endoplasmic reticulum, the microfilament network, the plasma membrane, vesicles and pits (Fig. 2b, arrows). The ciliary apparatus was constituted by a structurally typical basal body continuous with the finger-like ciliary shaft extending from the cell surface, and by an adjacent centriole orientated at right angles to the basal body (Fig. 2c, d and e). The microtubular arrangement was typical of non-motile cilia, which have a 9 + 0 microtubular pattern. Nine peripherally arranged triads of microtubules were present whereas the central pair was lacking (Fig. 2e and f). In appropriate section accessory structures were seen, such as the basal plate (Fig. 2c, black arrow), alar sheet (Fig. 2d and e) and ciliary rootlets (Fig. 2c and e, white arrows). See Fig. 2 legend for further description.

Much more rarely solitary cilia occurred at the luminal surface of the epididymal principal cells (Fig. 3). The ciliary shaft was directed into the lumen (Fig. 3a). Also in this case a 9 + 0 microtubular pattern was present (Fig. 3b).

4. Discussion

During recent years intensive studies have been focused on the primary cilium projecting from the surface of vertebrate cells, thus transforming it from a poorly understood curiosity into a structure recognized for its importance in development, inherited human disease and cancer. Cilia and flagella are ancient structures that have evolved in organisms as diverse as protozoa and vertebrates. It is now known that cilia are essential during development for the response to developmental signals, and evidence is accumulating that the primary cilium is specialized for hedgehog signal transduction, acting via the evolutionarily conserved mechanism of intraflagellar transport (Goetz and Anderson, 2010). Moreover, human disorders – called ciliopathies – have been described, affecting diverse organ systems and deriving on underlying structural or functional abnormalities of primary cilia (Tobin and Beales, 2009).

Apart from its involvement in differentiation and cell division, the primary cilium is known to be able to coordinate several essential cell signaling pathways, functioning as a dual sensor, mechanosensor and chemosensor (Singla and Reiter, 2006). The role of cilia in sensing the extracellular environment is best understood in the context of olfaction and photoreception. The innovative attribute of primary cilia as cellular *antennae* that sense a wide variety of signals might potentially take place in almost every vertebrate cell having a cell surface needing to be monitored, and a cross-talk to be performed.

There has been a persistent literature on primary cilia from the early 1960s, summarized in part by Wheatley (2005). Actually, primary cilia have been reported in many cell types of all the embryonic origin and differentiated localization in the body (Satir and Christensen, 2007). Although meticulously reviewed up to recent years by Bowser and Wheatley in the Primary Cilium Resource Page, no mention exists of the presence of primary cilia in the epithelial cells of the mammalian epididymis. Neither the presence of primary cilia has ever been reported in the literature specifically directed to studies on epididymis, to the best of our knowledge. On the contrary the interest on the functions, and correlated morphology, of the epididymal basal cell is still topical. Remarkable studies published in the last five years are still wondering about the possible role of this enigmatic cells type in the manifold functions of the epididymal epithelium.

According to the review by Cornwall (2009) basal cells – not accessing the luminal compartment – are in close association with the overlying principal cells and, by the presence of cytoplasmic extensions between principal cells may regulate its functions. Thus, basal cells are considered to regulate principal cell electrolyte transport by releasing paracrine factors. Moreover, cell–cell interactions within the epithelium can directly affect the luminal environment and ultimately sperm maturation. Recently, Shum et al. (2008) elegantly demonstrated that basal cells, previously believed to be