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COMMENTARY

The cell–cell junctions of mammalian testes—a summary

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The molecular and ultrastructural characteristics of the epithelial cells in the seminiferous tubules as well as the peritubular wall cells of mammalian testes have been subject of controversial debates and publications for several decades. Using biochemical as well as light and electron microscopical methods, in particular immunolocalization techniques, we have studied sexually mature testes from several mammalian species (man, bull, boar, guinea pig, rat, and mouse). The analyses included direct interspecies comparison of the tubular and peritubular structures with epithelial tissues of the adjacent excurrent duct system, including the epididymis, and in addition other comparative epithelial and muscular tissue controls (Domke 2018). The results of the subject studies have been published in a series of three articles "The cell-cell junctions of mammalian testes":

I. The adhering junctions of the seminiferous epithelium represent special differentiation structures (Domke et al. 2014).
II. The lamellar smooth muscle monolayer cells of the peritubular wall are laterally connected by vertical adherens junctions—a novel architectonic cell-cell junction system (Domke and Franke 2019).
III. Absence of an endothelial cell layer covering the peritubular wall of the seminiferous tubules—an immunocytochemical correction of a 50-year-old error in the literature (Franke et al. 2020).

I. The first study confirmed the presence of vimentin intermediate-sized filaments (IFs) and the absence of cytokeratin IFs in mature and spermiogenically active Sertoli cells. Furthermore, the molecular analyses of the corresponding cell–cell adhering junctions (AJs) of Sertoli cells with each other and with germ cells demonstrated the absence of desmosomes or "desmosome-like" junctions in contrast to the majority of references in the literature. In addition, epithelial molecules such as E-cadherin or EpCAM were also absent. In contrast, AJs present in the seminiferous tubules are based on AJs containing N-cadherin transmembrane molecules and cytoplasmic plaques containing α- and β-catenin, plakoglobin, proteins p120, and p0071 as well as a protein of the striatin family. These findings revealed that the intratubular Sertoli cells are interconnected with adjacent Sertoli cells as well as with germ cells by a novel type of AJs: Specific N-cadherin-based AJs, i.e., variously-sized, often very large cell–cell contacts (*areae adhaerentes*). In addition, certain regions of bovine Sertoli cells contain small clusters of sieve-like cell–cell contacts perforated by cytoplasm-to-cytoplasm channels 5–7 nm in luminal diameter (cribelliform junctions).

II. In continuation of our studies, we examined the well-developed basal lamina and the lamellar encasement structure, the peritubular walls, of the seminiferous tubules. We could show that the basal lamina is not attached to the Sertoli cells by any hemidesmosomal structures and molecules. The peritubular walls are composed of a bandage system of closely packed monolayers of very thin lamellar smooth muscle cells (LSMCs) interspersed with layers of extracellular matrix (ECM). The number of these lamellar monolayers can vary from one to six per bandage system in different species, developmental stages, and regions. Their smooth muscle cell (SMC)-specificity is indicated by the content of smooth muscle α-actin, the corresponding myosin light and heavy chains, tropomyosin, α-actinin, smoothelin, desmin, vimentin, talin, filamin A, dystrophin, drebrin, caveolin, caldesmon, calponin, and protein SM22α. In addition, the peritubular SMC cytoplasm is enriched with myofilament bundles and other typical

This commentary is dedicated to Werner W. Franke on the occasion of his 80th birthday.

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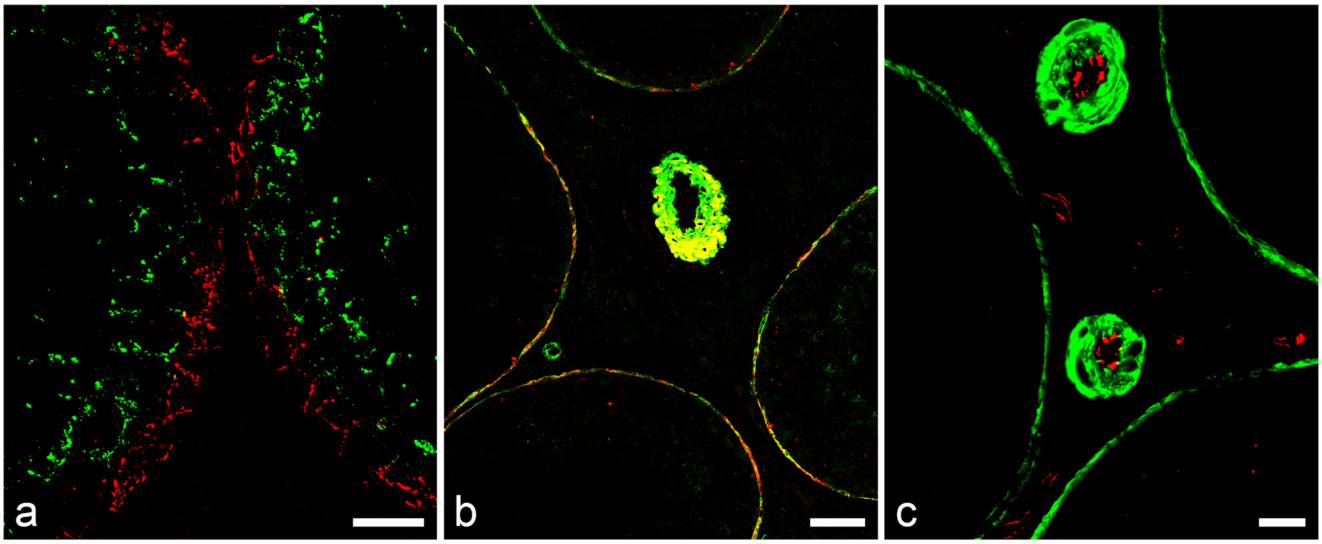

Fig. 1 Double-label immunofluorescence microscopy of cryostat cross-sections through seminiferous tubules of frozen bull testes. (**a**) Immunostaining reactions with antibodies against N-cadherin (green) are exclusively present in cell-cell junctions of the Sertoli cells and spermatogonia within the seminiferous tubules. Immunoreactions of E-cadherin-containing junctions (red) are seen only in a special layer of the peritubular wall (Domke et al. 2014). (**b**) The peritubular lamellar smooth muscle cells (LSMCs) around the seminiferous tubules and the perivascular smooth muscle cell (SMC) layers of a blood vessel show immunostaining reaction with antibodies against SMC markers smoothelin (red) and desmin (green; Domke and Franke 2019). (**c**) Immunostaining reactions are shown with antibodies against SMC marker desmin (green) and endothelial marker VE-cadherin (red). The peritubular LSMCs as well as blood vessel cells are positive for the SMC marker but negative for the endothelial marker (Franke et al. 2020). Bars (a) 20 μm, (b) 50 μm, (c) 20 μm

SMC structures such as "dense bodies," plasma membrane-associated "focal adhesions," and caveolae. Hence, these LSMCs are a novel kind of smooth muscle cells and tissue and not just as they have been described in the literature as "myoid cells," "myofibroblasts," or "myoepithelial cells." Furthermore, these LSMCs are connected by end-to-end junctional contacts and laterally by numerous, vertical AJs located in overlapping cell processes (*alter super alterum*). These AJs are based on cadherin-11, often in addition with P-cadherin in some species, and are anchored in cytoplasmic plaques formed by β-catenin, plakoglobin, occasionally protein p0071, and also the 54 kDa plaque protein myozap as well as protein LUMA.

III. In our third article, we could show that the peritubular wall structure of all species examined is not surrounded by a continuous lymphatic endothelium as it has often been claimed in the literature, in particular for rodent testes. Hence, endothelial cell type marker molecules such as VE-cadherin, claudin-5, protein PE-CAM (CD31), protein LYVE-1, and podoplanin are absent in the monolayer cells covering the peritubular wall. (For a highlight of each article see Fig. 1).

In this article series, we discuss possible methodological reasons for the maintenance of incorrect cell type classifications in the literature and emphasize the value of molecular analyses using multiple cell type-specific markers, also with respect to physiology and medical sciences.

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