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Testicular-borne factors affect sperm fertility:

Immature testicular germ cells secrete factors that influence sperm maturation in mice

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Male fertility relies on the genesis of sperm in seminiferous tubules of the testis and their maturation during transit through the epididymis. Mouse models with impaired development of the most proximal region of the epididymis, the initial segment (IS), possess sperm that are morphologically normal but incapable of fertilizing an egg (1). It has been postulated that factors synthesized in the testis are released into the lumen of tubules (lumicrine factors) and influence development and function of the IS. However, the identity of such factors has remained elusive. On page 1132 of this issue, Kiyozumi *et al.* (2) identify and characterize the first known lumicrine factor, the germ cell-secreted protein neural epidermal growth factor-like like 2 (NELL2). They demonstrate a key role for NELL2 in driving development of the IS of mice, culminating in the production of IS-secreted proteases that are indispensable for sperm processing in the epididymis and thus male fertility.

The epididymis is a convoluted ductal system of up to 1 m in length in mice and 6 m in humans. Testicular sperm must transit the length of the epididymis to gain the capacity for fertilization, including activation of the machinery that drives motility as well as egg recognition and binding. Given that spermatozoa are transcriptionally and translationally silent, the process of sperm maturation is largely attributed to activities of epididymal epithelial cells, which can include post-translational modification, augmentation, and processing of sperm proteins. Proteomic comparisons of sperm collected from the more proximal regions of the epididymis (caput) with sperm from the distal region (cauda) reveal differential expression in more than 50 proteins (3) as well as 77 phospho-protein changes (4).

Given the importance of the epididymis in sperm maturation, aberrations in epididymal function can be catastrophic for male fertility. Although endocrine factors such as androgens are integral for epididymal development during embryonic and early postnatal life, studies with mice indicate that lumicrine factors are critical for differentiation of the IS from postnatal day 15 (P15) to P19 (5). Indeed, when the efferent ductules that connect testicular seminiferous tubules and epididymal tubules are ligated during development, thus preventing

the flow of lumicrine factors into the epididymis, the epithelial cells of the IS do not undergo differentiation and default into an apoptotic pathway (6, 7). Although testicular lumicrine factors likely stimulate IS differentiation and proliferation through activation of SFKs (SRC proto-oncogene family kinases), ERKs (extracellular signal-regulated kinases), and AMPKs (adenosine mono-phosphate-activated protein kinases) within the epithelial cells (5), the identity of these lumicrine factors has remained elusive.

In 1999, an orphan receptor tyrosine kinase, c-ros oncogene 1 (ROS1), was identified as the first putative lumicrine receptor that is expressed in the IS of the mouse epididymis (1). Although spermatogenesis was grossly normal, development of the IS in *Ros1*-deleted mice was impaired, and their sperm were unable to fertilize an egg. This led to the hypothesis that factors produced in the postnatal testis transit to the epididymis to stimulate differentiation and development of the IS and drive epithelial cells to produce molecules essential for sperm maturation. Unanswered questions center on which testicular cell types (germ cell and/or somatic cell) are responsible for synthesis of lumicrine factors, what these factors are, and how they stimulate the epithelial cells of the IS to differentiate and influence sperm maturation.

Kiyozumi *et al.* provide evidence that lumicrine factors that drive IS development are produced by testicular germ cells (which produce spermatozoa). They find that NELL2 binds directly to the ROS1 receptor, and inactivation of the *Nell2* gene phenocopies the *Ros1*-deleted mice. Specifically, *Nell2*-deleted mice have a poorly differentiated IS and produce sperm that cannot transit the uterotubal junction in the female reproductive tract or bind the glycoprotein “shell” (called the zona pellucida) that surrounds the oocyte, thus culminating in infertility.

Kiyozumi *et al.* highlight that impaired IS development attributed to lumicrine interruption becomes appreciable from 2 to 3 weeks of age, coinciding with the appearance of the first spermatocytes in the testis. Mining testicular single-cell RNA-sequencing databases revealed that *Nell2* expression is indeed restricted to spermatocytes in adult mice. To consolidate the idea that spermatocyte-produced factors influence IS development, the authors demonstrate a previously unappreciated immaturity of the IS in genetically sterile mice that lack advanced germ cells. Together, these data provide compelling evidence for a previously undetermined interaction between germ cells and the somatic cells that line the epididymis and regulate sperm maturation (see the figure).

To understand how lumicrine factors prepare the IS epithelial cells to participate in sperm maturation, Kiyozumi *et al.* investigated the molecular events that follow NELL2-ROS1 binding. They identified the uncharacterized protease ovochymase 2 (OVCH2) that is secreted by the IS epithelial cells to cleave and thus activate a key protein involved in sperm-egg binding [A disintegrin and metallopeptidase domain 3 (ADAM3)]. Thus, as is the case with *Nell2*-, *Ros1*-, and *Adam3*-deleted mice, *Ovch2*-deleted mice produced morphologically normal sperm that are unable to transit the uterotubal junction in the female reproductive tract or bind the egg zona pellucida for fertilization.

The NELL2-ROS1-OVCH2-ADAM3 pathway characterized by Kiyozumi *et al.* is an eloquent example of lumicrine function that is likely to catalyze characterization of other similar molecular cross-talk networks that regulate male fertility. Certainly, it is possible that OVCH2 cleaves other key proteins in the spermatozoon that aid in its ascent to become fertilization competent. Further, it is likely that other lumicrine factors are at play to stimulate epididymal maturation and the secretion of alternate protein processing enzymes that drive sperm maturation during epididymal transit.

The characterization of lumicrine signaling between the germ cells and epididymis could profoundly alter the understanding of male infertility and, further, stimulate the development of new approaches for treating infertility or devising nonhormonal male contraceptives. In up to 80% of male infertility cases, sufficient numbers of sperm are produced, yet functionality is compromised (8, 9). A major cause of such fertilization failures is an inability of the sperm to recognize and bind to the zona pellucida (10), similar to the phenotype of lumicrine-deficient mouse models. Given that the causes of up to 60% of male infertility cases are unknown (8), exploring defective “lumicrinology” will provide a new avenue for diagnosis and treatment. Moreover, targeting the lumicrine system may present an efficacious strategy for identifying druggable targets for the long sought-after male contraceptive pill.

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figure. Lumicrine regulation of epididymal development and function

Lumicrine factors, such as neural epidermal growth factor–like like 2 (NELL2), are produced by germ cells in the testis during maturation of the epithelial cells that line the initial segment (IS) of the epididymis through activation of c-ros oncogene 1 (ROS1). Mature IS cells produce ovoidysmase 2 (OVCH2), which cleaves a disintegrin and metalloproteinase domain 3 (ADAM3) on immature sperm to attain fertilization competency.