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RESEARCH HIGHLIGHT

Hidden gems in the niche: a new approach to the study of spermatogonial stem cells

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Kanatsu-Shinohara and colleagues continue to reveal the secrets of the rare mammalian spermatogonial stem cells. Their most recent study offers a new approach by applying principles from hematopoietic stem cell research to demonstrate that cells which form a cobblestone-like underlay beneath testicular somatic cells in culture are spermatogonial stem cells. Utilization of mouse models and cell cultures shows how the chemokine, CXCL12, fits into the signalling cascade which governs the fate of these cells and hence is essential to male fertility.

Many differentiated but renewable cell types in the body are derived from relatively small populations of dedicated precursor cells, or stem cells, which maintain an essentially unlimited capacity for continued divisions.[1,2] The existence in mammalian testes of spermatogonial stem cells (SSCs), which are the foundation of spermatogenesis and essential for adult male fertility, has been well recognized.[3] However, unlike in *Drosophila* testes, the whereabouts of the mammalian SSC niche has been difficult to determine. Definitively identifying SSCs and uncovering the nature of the niche biology has been a critical challenge for the field.

The pioneering work of Ralph Brinster and his colleagues demonstrated that SSCs can be defined by their capacity to form colonies that reconstitute full spermatogenesis *in vivo*,[4,5] a cumbersome yet unequivocal assay that mirrors the strategy used to identify stem cells in other systems and particularly in hematopoiesis. This approach taught us that SSCs can be grown in culture for many passages, stored in the freezer and used to reverse infertility arising from genetic defects and that they are most abundant in the newborn testis at the onset of spermatogenesis. These clues have given researchers tools with which to pursue an understanding of the signalling pathways that control their proliferation and survival. Key findings include the demonstration that integrins β1 and α6 are present on the SSC surface, while Kit is not,[6] illuminating the progression of markers and communication molecules that are essential for normal spermatogenesis.

The whereabouts of the niche is more ephemeral, with Yoshida and colleagues[7] hypothesizing that the SSC niche is created by the proximity of the adjacent vasculature to the basement membrane of the seminiferous tubule and concomitant influence of extra-testicular factors such as retinoic acid.[8] However, major hurdles still exist regarding identification of the true niche and pinpointing what we expect is a complicated set of interacting factors that govern SSC fate and plasticity *in vivo*. The recent publication by Kanatsu-Shinohara and colleagues[9] in *Cell Stem Cell* provides an important way forward.

As the title highlights, this study provides a new approach to stem cell identification, with convincing evidence that these newly described cobblestone colonies are a robust indicator of spermatogonial stem cell prevalence in testis cell cultures. Interactions between somatic and germ cells are enabled in the culture system which uses a feeder layer of non-dividing somatic cells plated on a laminin-coated culture dish onto which germline cells are introduced. Cell clusters form both above and below the feeder layer, and were viewed in this study with the aid of a green fluorescent protein tag present in the introduced cells to mark spermatogonia. Recognition of clusters of flat cells growing underneath the feeder layers, described as similar in appearance to the cobblestone colonies of hematopoietic cell clusters, enabled these researchers to isolate a distinct subfraction of spermatogonia which they used in the now classical transplantation assays to demonstrate their capacity to function as SSCs. Thus, building on knowledge derived from analysis of hematopoiesis and the growth characteristics which control hematopoietic stem cells, Kanatsu-Shinohara and colleagues[9] offer a new avenue by which the factors that control spermatogonial stem cell biology can be interrogated.

This study contains several important extensions of knowledge that has been accumulating in the field regarding SSC behavior. The functional importance of the chemokine, CXCL12, and its relationship to the essential SSC growth factor, glial cell line-derived neurotrophic factor (GDNF) was explored in a series of experiments. These included cell culture experiments to address what stimulates CXCL12 production by testicular cells. The combinatorial influence of epidermal growth factor, fibroblast growth factor, GDNF and follicle-stimulating hormone was demonstrated using this simple, yet valuable approach which demonstrates the multifactorial nature of signaling inputs which govern developmental switches in germline genesis.

Elegant experiments show that there are distinct roles for GDNF or CXCL12 when SSCs are exposed to elevated and reduced levels of these *in vivo*. Intact signaling by either ligand stimulated pathway is essential for SSC function in transplantation assays, and this directly corresponds with the capacity for cobblestone colonies to form in culture. In addition, colonies formation in recipient testes after transplantation was directly related to the level of CXCL12 signaling, implicating CXCL12 in SSC homing to the niche *in vivo*.

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How can these new findings lead to developments that will aid treatment of men with infertility or elucidate the underpinnings of testicular cancer? While there are clues to what controls GDNF[10] and CXCL12 synthesis provided in this analysis, the mechanisms by which chemokine release is regulated are not known. Does the upregulation of CXCR4 expression in gonocytes rely exclusively on GDNF activation of the transcription factor ETV5?[11] Is aberrant signaling by CXCR4 implicated in the onset of testicular cancer?[12]

Are other chemokines, growth factors and receptors involved in homing, as demonstrated previously for extracellular matrix stem cell factor (Kit Ligand) in guiding the migration of primordial germ cells to the indifferent genital ridge during murine embryogenesis?[13] Does the CXCL12/CXCR4 axis control the first wave of spermatogenesis, events in normal adult homeostasis, or the extraordinary events that relate to transplantation assays in which whole cohorts of germline depletion create an artificially barren epithelium? What is the role of the alternate CXCL12 receptor CXCR7 in creating the chemokine gradient?

Important information comparing human and rodent models is outstanding that will help us understand whether the triggers for SSC differentiation at the onset of spermatogenesis immediately after birth are common to events in the human when spermatogonia first appear in fetal life. This knowledge will indicate whether the factors underpinning development of carcinoma *in situ* that prevent normal germline differentiation can be used to drive therapeutic differentiation of cells that would otherwise become carcinogenic in testis cancer patients. The potential to offer hope to infertile men who have stem cells but do not make sperm will be enriched by a better understanding of the critical signals from the SSC niche which allow these cells to be sustained and to multiply.

1 Becker AJ, Mc CE, Till JE. Cytological demonstration of the clonal nature of spleen colonies derived from transplanted mouse marrow cells. *Nature* 1963; **197**: 452–4.
2 Siminovitch L, McCulloch EA, Till JE. The distribution of colony-forming cells among spleen colonies. *J Cell Physiol* 1963; **62**: 327–36.
3 de Rooij DG, Russell LD. All you wanted to know about spermatogonia but were afraid to ask. *J Androl* 2000; **21**: 776–98.
4 Oatley JM, Brinster RL. The germline stem cell niche unit in mammalian testes. *Physiol Rev* 2012; **92**: 577–95.
5 Brinster RL. Germline stem cell transplantation and transgenesis. *Science* 2002; **296**: 2174–6.
6 Shinohara T, Avarbock MR, Brinster RL. beta1- and alpha6-integrin are surface markers on mouse spermatogonial stem cells. *Proc Natl Acad Sci U S A* 1999; **96**: 5504–9.
7 Yoshida S, Sukeno M, Nabeshima Y. A vasculature-associated niche for undifferentiated spermatogonia in the mouse testis. *Science* 2007; **317**: 1722–6.
8 Sugimoto R, Nabeshima Y, Yoshida S. Retinoic acid metabolism links the periodical differentiation of germ cells with the cycle of Sertoli cells in mouse seminiferous epithelium. *Mech Dev* 2012; **128**: 610–24.
9 Kanatsu-Shinohara M, Inoue K, Takashima S, Takehashi M, Ogonuki N *et al*. Reconstitution of mouse spermatogonial stem cell niches in culture. *Cell Stem Cell* 2012; **11**: 567–78.
10 Sato T, Aiyama Y, Ishii-Inagaki M, Hara K, Tsunekawa N *et al*. Cyclical and patch-like GDNF distribution along the basal surface of Sertoli cells in mouse and hamster testes. *PLoS One* 2011; **6**: e28367.
11 Wu X, Goodyear SM, Tobias JW, Avarbock MR, Brinster RL. Spermatogonial stem cell self-renewal requires ETV5-mediated downstream activation of Brachyury in mice. *Biol Reprod* 2011; **85**: 1114–23.
12 Gilbert DC, Chandler I, McIntyre A, Goddard NC, Gabe R *et al*. Clinical and biological significance of CXCL12 and CXCR4 expression in adult testes and germ cell tumours of adults and adolescents. *J Pathol* 2009; **217**: 94–102.
13 Gu Y, Runyan C, Shoemaker A, Surani A, Wylie C. Steel factor controls primordial germ cell survival and motility from the time of their specification in the allantois, and provides a continuous niche throughout their migration. *Development* 2009; **136**: 1295–303.