The New Director of "the Spermatogonial Niche": Introducing the Peritubular Macrophage

Marvin L. Meistrich[1,*] and Gunapala Shetty[1]
[1]Department of Experimental Radiation Oncology, University of Texas MD Anderson Cancer Center, TX 77030, USA
*Correspondence: meistrich@mdanderson.org
http://dx.doi.org/10.1016/j.celrep.2015.07.057

In this issue of *Cell Reports*, DeFalco et al. (2015) characterize a novel macrophage population associated with the peritubular lamina of mouse testes. These macrophages may create a niche not for the self-renewal of stem cells but rather the induction of their differentiation.

Identification of a spermatogonial niche in mammals has been elusive. In *Drosophila* and *Caenorhabditis elegans*, the tube-shaped gonads have polarity, and there is a well-defined spermatogonial stem cell (SSC) niche at the closed end. In contrast, mammalian seminiferous tubules are connected at both ends to the rete testis and do not appear to have polarity. SSCs are in the basal region of the seminiferous tubules, which is bounded by Sertoli and peritubular myoid cells and the extracellular matrix forming the basal lamina (Figure 1). The tubules appear circumferentially symmetric and invariant along their length except for the wave of stages of the cycle of the seminiferous epithelium of germ cells. However, the persistence of the stem cells means that they must be present in tubules at all stages. Several molecules required for stem spermatogonial self-renewal and maintenance, GDNF and chemokines (e.g., CXCL12), are produced uniformly by all Sertoli cells (Yang et al., 2013). The uniformity of structure and factors has raised questions as to whether there are defined niches or just a random distribution of SSCs along the basal lamina.

The SSCs in many mammals are morphologically classified as undifferentiated type A spermatogonia. Most of the stem cell potential lies within the A_{single} subpopulation, which is not connected to other spermatogonia by intercellular bridges. Mouse testis tubules contain about 3,000 SSCs (Nagano, 2003) among the ~30,000 A_{single} spermatogonia, which are scattered along the 2,000 mm^2 of seminiferous tubule basal lamina (Russell et al., 1990). When the undifferentiated A_{single} spermatogonia divide, they can self-renew or produce chains of $A_{aligned}$ spermatogonia, which still appear morphologically undifferentiated but lose expression of some stem cell markers and acquire differentiation markers. These cells generally have lost their stem cell potential (Yoshida, 2012). Subsequently, retinoid stimulation induces the $A_{aligned}$ to transform into type A_1 differentiated spermatogonia and continue on a tightly regulated differentiation sequence.

The distribution of the undifferentiated type A spermatogonia is affected by only one asymmetrical feature of the seminiferous tubule. Some basal regions are adjacent to other tubules, whereas other regions are adjacent to interstitial regions comprised of vasculature, Leydig cells, and interstitial macrophages. $A_{aligned}$ spermatogonia are preferentially associated with these vascular interstitial areas (Yoshida et al., 2007). However, the exact source (vascular, Leydig, or macrophage) of additional factor(s) regulating the spermatogonial events in their vicinity is not known. Furthermore, it is still unclear whether the A_{single} stem cells are also preferentially localized in this region.

Studies of macrophages in the testis have focused on those associated with Leydig cells and vascular elements in the interstitium (Hume et al., 1984; Hutson, 2006). In this issue of *Cell Reports*, DeFalco et al. (2015) beautifully describe a different macrophage population located at the surface of seminiferous tubules in the adult mouse testis that they propose contributes to a spermatogonial niche. These peritubular macrophages have small cell bodies and multiple dendritic-like processes and are intermingled with the peritubular myoid cells and associated with blood vessels. They are positive for seven macrophage markers tested by immunofluorescence but differ from the interstitial macrophages in the ratio of CSF1R and MHCII expression.

Interestingly, quantification of the numbers of peritubular macrophages shows that they are similar in number to the A_{single} spermatogonia. Furthermore, there is a correlation between macrophage density and numbers of stem through early differentiating spermatogonia in tubules at different stages of the seminiferous epithelial cycle. However, they never report the distances between the peritubular macrophages and spermatogonia to quantify whether or not there is a significant association between the two cell types. Nevertheless, in their most important observation, they conclusively show that selective reduction of the numbers of macrophages, including the novel ones in the peritubular region, results in a decline in the numbers of $A_{aligned}$ spermatogonia without a significant effect on the A_{single} cells. The degree of decline of the $A_{aligned}$ cells is correlated with the localized degree of loss of peritubular macrophages. Thus, macrophages, and likely the peritubular ones, control an early step of differentiation of the stem cell.

Among macrophage-produced factors, colony-stimulating factor 1 (CSF1) had been shown to enhance expansion of cultured SSC in vitro, although its activity has not been demonstrated in vivo. DeFalco et al. (2015) demonstrate that both peritubular and interstitial macrophages produce CSF1, suggesting a mechanism by which macrophages act on SSCs. It should be noted that this result disagrees with an earlier report (Oatley et al., 2009)

that Leydig cells and "select peritubular myoid" cells express CSF1; however, the apparent select peritubular myoid cells observed previously could very well have been the peritubular macrophages reported here. Additional investigation of whether CSF1 may be one of the factors signaling SSC self-renewal or differentiation should be pursued.

In conclusion, there may not be a niche specifying the location of SSCs in any specific part of the basal region of the seminiferous tubule. The early A_{single} and $A_{aligned}$ spermatogonia, as shown with Gfra1-GFP marked cells, are motile (Hara et al., 2014). Perhaps when they move to regions of tubules adjacent to vascular and interstitial complexes and/or peritubular macrophages, they are induced to initiate differentiation, as shown by expression of Ngn3-GFP, and have reduced motility. Instead of there being a stem cell niche, there appears to be a niche for induction of differentiation. Perhaps the initial step of dividing to form $A_{aligned}$ is due to the migration of spermatogonium into the differentiation niche. If they are not in such a region, then they are more likely to divide into two stem cells, providing a mechanism for self-renewal.

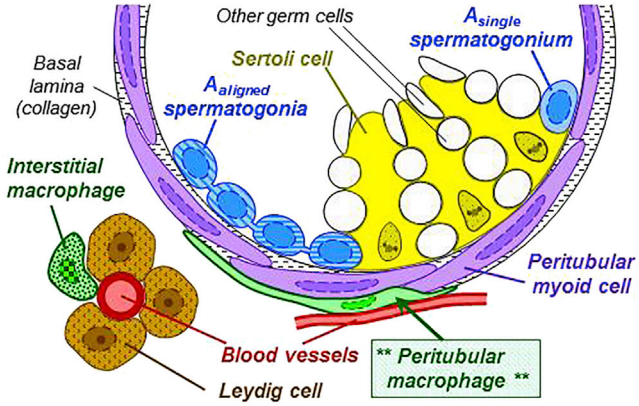

Figure 1. Diagram of Portion of a Seminiferous Tubule and Surrounding Somatic Cells

The stem cells are a subpopulation of the morphologically undifferentiated A_{single} spermatogonia. The critical step in stem cell differentiation occurs during the formation of pairs and chains of morphologically undifferentiated A spermatogonia ($A_{aligned}$). This process preferentially occurs in regions of the tubule adjacent to clusters of interstitial macrophages and Leydig cells around blood vessels. DeFalco et al. (2015) describe a novel class of macrophages (**) associated with the peritubular region and whose local presence is required for the differentiation of A_{single} into $A_{aligned}$ spermatogonia. Thus, although there may not be a specific niche for stem cells within the basal region of the tubules, peritubular macrophages may contribute to defining a niche for initiation of differentiation.

REFERENCES

DeFalco, T., Potter, S.J., Williams, A.V., Waller, B., Kan, M.J., and Capel, B. (2015). Cell Rep. *12*, this issue, 1107–1119.

Hara, K., Nakagawa, T., Enomoto, H., Suzuki, M., Yamamoto, M., Simons, B.D., and Yoshida, S. (2014). Cell Stem Cell *14*, 658–672.

Hume, D.A., Halpin, D., Charlton, H., and Gordon, S. (1984). Proc. Natl. Acad. Sci. USA *81*, 4174–4177.

Hutson, J.C. (2006). Exp. Biol. Med. (Maywood) *231*, 1–7.

Nagano, M.C. (2003). Biol. Reprod. *69*, 701–707.

Oatley, J.M., Oatley, M.J., Avarbock, M.R., Tobias, J.W., and Brinster, R.L. (2009). Development *136*, 1191–1199.

Russell, L.D., Ettlin, R.A., Hikim, A.P.S., and Clegg, E.D. (1990). Histological and Histopathological Evaluation of the Testis (Clearwater, FL: Cache River Press).

Yang, Q.E., Kim, D., Kaucher, A., Oatley, M.J., and Oatley, J.M. (2013). J. Cell Sci. *126*, 1009–1020.

Yoshida, S. (2012). Reproduction *144*, 293–302.

Yoshida, S., Sukeno, M., and Nabeshima, Y. (2007). Science *317*, 1722–1726.