analyses showed that GSCs expressed all of these molecules except *Cldn5* (Figure 6A). In contrast, W testis lacked expression of *Cldn9*, *13*, *14*, *16*, *17*, *18*, *21*, and *24*. Using real-time PCR, we then quantitatively assessed the expression levels of these commonly expressed genes in control and DN-Rac cells (Figure 6B). Although we found no significant changes in *Ocln* or *Cldn1*, *2*, *10b*, *11*, *12*, *19*, *20*, or *22* expression, *Cldn3*, *4*, *6*, *7*, *8*, *10a*, *15*, and *23* were downregulated in DN-Rac cells. These cells also showed normal expression of *Etv5*, which regulates claudin5 expression and is involved in SSC self-renewal (Morrow et al., 2009). Of the differentially regulated claudins, western blotting showed reduced expression of Cldn3, 7, and 8 in DN-Rac cells. Ocln and Cldn10 expression, however, were not significantly altered (Figure 6C). While Cldn4, 6, and 15 were not expressed in either GSCs or W testis at the protein level, Cldn11 and 23 were found only in the W testis. Immunohistological staining confirmed the expression of Cldn3, 7, and 8 in spermatogonia in vivo (Figure 6D). While Cldn3 and 8 were expressed in cells on the basement membrane, Cldn7 was more widely expressed in the seminiferous tubules.

Functional Analyses of Tight Junction-Associated Proteins by Germ Cell Transplantation

In the final set of experiments, we investigated the impact of these candidate tight junction-associated molecules on SSC homing. We first evaluated the SSC activities of *Ocln* and *Cldn11* KO testis cells (Figure 6E). While *Ocln* KO mice gradually lose spermatogenesis and become infertile, *Cldn11* KO mice lack Sertoli cell tight junction strands and spermatozoa are never observed (Gow et al., 1999; Saitou et al., 2000). In two experiments, $\sim 4 \times 10^5$ adult mutant testis cells were microinjected into the seminiferous tubules of adult W mice. Three months after transplantation, we found normal-appearing spermatogenesis from both mutant donors. In experiments with *Ocln* KO mice, 18.8% ± 1.7% tubules showed spermatogenesis with control cells, and 18.3% ± 2.7% (n = 6) tubules showed spermatogenesis with mutant cells. Likewise, in experiments with *Cldn11* KO mice, 4.7% ± 1.6% tubules showed spermatogenesis with control cells, and 6.2% ± 2.0% (n = 6) tubules showed spermatogenesis with mutant cells. Both *Ocln* and *Cldn11* KO SSCs reinitiated spermatogenesis with normal appearance (Figure S4A), and no significant differences were found in both experiments. These results indicate that these mutant SSCs can migrate through the BTB and reinitiate spermatogenesis as long as tight junction proteins are available in the host environment.

We further evaluated the functions of claudin3, 7, and 8 that were downregulated in DN-Rac cells. To examine the effects of claudin downregulation, we suppressed endogenous expression of claudins by delivering shRNAs via lentiviruses (Figure S4B and Table S6). ROSA GSCs were transduced with claudin knockdown (KD) vectors, and shRNA against EGFP was used as a control. Four days after infection, $\sim 4 \times 10^3$ cells were microinjected into the seminiferous tubules of adult W mice. Claudin3 KD by shRNA significantly reduced ROSA26 GSC colonization in three experiments (Figure 6F). Although claudin7 and 8 KD also decreased colonization, the differences were not significant. Claudin shRNA treatment did not enhance apoptosis of GSCs (Figure S4C). Taken together, these results indicate that claudin3 is involved in the SSC transmigration through the BTB after transplantation.

DISCUSSION

Rac is involved in many biological processes, including cell migration and regulation of cell shape, by eliciting actin polymerization at the plasma membrane to produce lamellipodia and membrane ruffles (Burridge and Wennerberg, 2004). The involvement of Rac in stem cell homing was previously demonstrated for HSCs (Cancelas et al., 2006). While *Rac2*-deficient mice showed defects in long-term engraftment, *Rac1*-deficient HSCs were incapable of short-term engraftment. Interestingly, loss of both *Rac1* and *Rac2* led to massive mobilization of HSCs from the bone marrow. Our study shows that spermatogonia express *Rac1* predominantly, and this is the second molecule demonstrated to be involved in SSC homing. Unlike β1-integrin, which mediates attachment of SSCs to the basement membrane, our results suggest that Rac1 operates in a different step of SSC homing by regulating the transmigration of SSCs through the BTB.

The migration of SSCs through tight junctions at the BTB is a striking feature of the homing process. While preleptotene spermatocytes migrate adluminally from the basal side of the seminiferous tubules during normal spermatogenesis, transplanted SSCs migrate in the opposite direction toward the germline niche on the basement membrane. The ability of transmigration is not limited to spermatogonia in the postnatal testis; prospermatogonia in the fetus as early as 13.5 days postcoitum, which lack expression of spermatogonia markers, can also colonize adult seminiferous tubules (Ohta et al., 2004), indicating that homing ability is an important characteristic acquired in the early stages of development. To successfully cross the BTB, SSCs must bypass or block the tight junctions between Sertoli cells, which consist of claudins or occludin. Not surprisingly, this process is inefficient because <10% of the transplanted SSCs can reinitiate spermatogenesis after transplantation into the adult testes (Oatley and Brinster, 2008). On the other hand, the transplantation efficiency increased by about 10-fold when SSCs were transplanted into pup testes without a BTB, indicating that this is the most important step of SSC homing (Shinohara et al., 2001). However, the early phases of SSC colonization are difficult to study because no SSC-specific markers exist, and by definition, SSCs are detected only after producing germ cell colonies. In this study, the involvement of Rac1 in transmigration was examined using pup and adult recipients for transplantation. While *Itgb1*-deficient SSCs disappeared within 3 weeks post-transplantation in either pup or adult testes (Kanatsu-Shinohara et al., 2008), *Rac1*-deficient SSCs could colonize pup, but not adult, seminiferous tubules, indicating that the condition of the host testes is important in Rac-mediated SSC homing.

Spermatogonia are known to express several tight junction proteins as well as adherens junction proteins, despite the apparent lack of either type of junction (Kanatsu-Shinohara et al., 2006; Morrow et al., 2009). They may nonetheless play important roles in spermatogenesis, because loss of occludin causes spermatogenic failure and male infertility (Saitou et al., 2000). Moreover, *Cldn11* KO mice lose tight junction intramembranous strands in Sertoli cells, and are sterile (Gow et al., 1999).

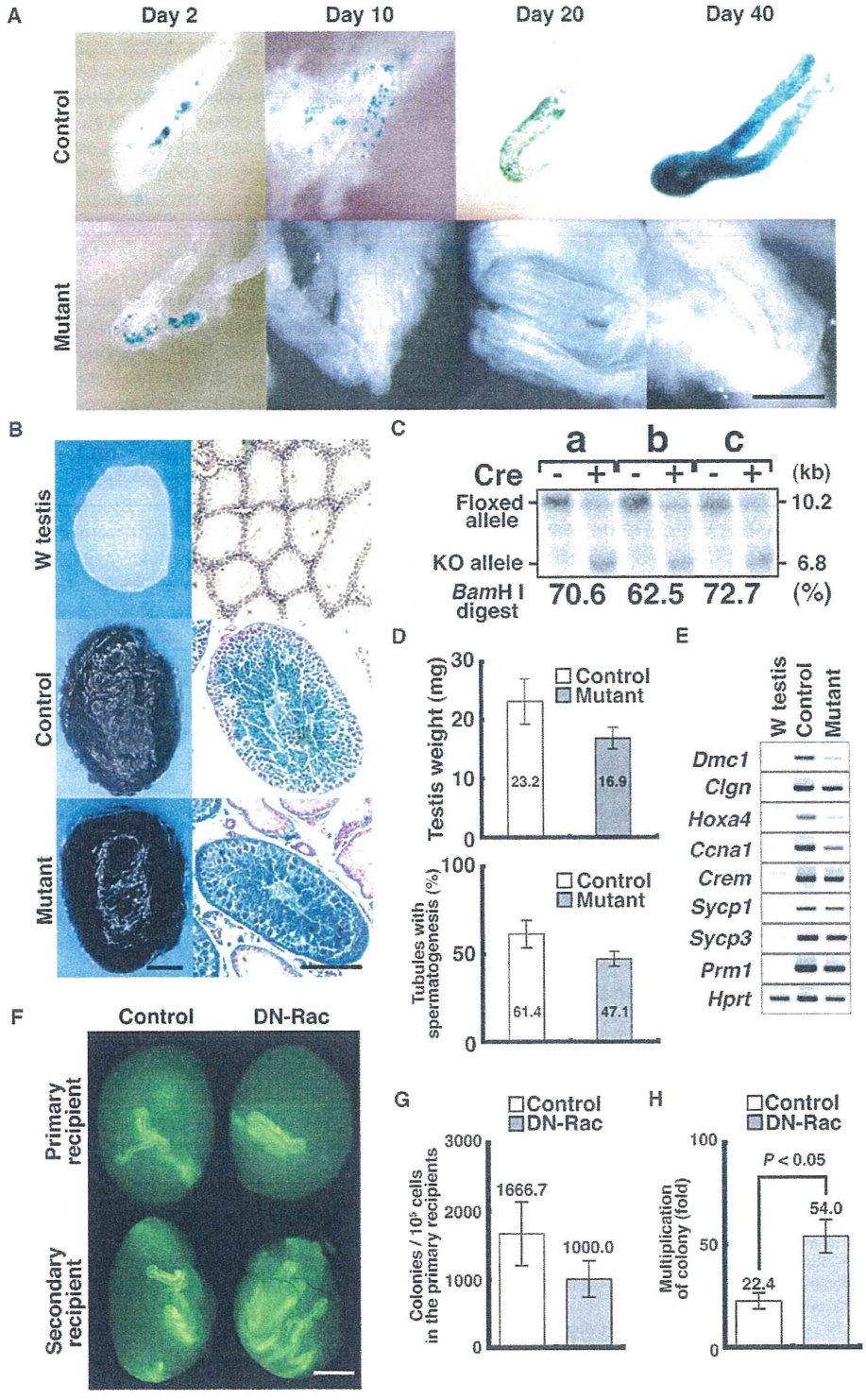

Figure 5. Colonization of Rac1 Mutant Cells in Immature Pup Testis

(A) Colonization of adult recipient mouse seminiferous tubules that received Cre-treated homozygous mutant and control testis cells. Although mutant cells were found at 2 days posttransplantation, they disappeared rapidly after 10 days. In contrast, control cells formed networks of spermatogonia at 20 days. Colonies with an intense blue color were found at 40 days, indicating multiple layers of germ cells.

Claudin expression in Sertoli cells is under complex regulation, because androgens change the permeability of the BTB by regulating claudin3 expression (Meng et al., 2005). Furthermore, claudin5 expression increases when spermatogenesis is established after transplantation in W mice (Morrow et al., 2009). Although whether infertility phenotypes in these mice are caused by abnormalities in germ cells or Sertoli cells is unclear, tight junction proteins on both germ cells and Sertoli cells are likely regulated dynamically and abnormalities in this interaction cause infertility or homing defects (Figure 7).

The results of the present study indicate that several claudins were downregulated in DN-Rac GSCs. To our knowledge, this is the first report showing the regulation of claudins by Rac. Although most of the claudins are expressed in GSCs, not all of them were translated into proteins, and functional analyses of these claudins revealed that claudin3 is involved in the transmigration of SSCs. Claudin3 is unique because it is transiently associated with newly formed tight junctions at the time when germ cells move from the basal to the adluminal compartment during stages VIII–IX of the seminiferous epithelial cycle (Meng et al., 2005). This is in contrast with claudin11 and occludin, which remain associated with the tight junction during the entire cycle of the seminiferous epithelium. In the transfer of spermatocytes to the adluminal compartment, Sertoli cells were previously postulated to play an active role and a transient intermediate compartment was thought to be created, thereby sealing these cells off from the rest of the seminiferous epithelium (Russell, 1977). This would accommodate the passage of germ cells without disrupting the integrity of the BTB. The validity of this model, however, has remained controversial (Mruk and Cheng, 2004). Whether SSCs create a similar compartment when they move to the opposite direction and how many different kinds of claudins on Sertoli cells comprise the BTB remain unknown. We speculate that SSCs and preleptotene spermatocytes may use different combinations of claudins for transmigration because *Rac1* KO SSCs underwent normal spermatogenesis in the pup recipients when the animals matured into adults with a BTB. Further analyses using the germ cell transplantation technique will provide insights into the mechanism of this unique biological process.

On the other hand, Rac1 is dispensable in tethering SSCs to the niche on the basement membrane. Because Rac is often activated downstream of integrin receptors and Rac deficiency causes mobilization of HSCs from bone marrow (Burridge and Wennerberg, 2004; Cancelas et al., 2006), we expected that Rac would reinforce integrin-mediated signaling and that *Rac1*-deficient cells would exhibit a phenotype similar to that of *Itgb1*-deficient SSCs. Unexpectedly, *Rac1* KO SSCs and DN-Rac cells were able to bind to the basement membrane and laminin-coated plates, respectively, indicating that Rac1 is dispensable for integrin-mediated adhesion. Enhanced adhesion of DN-Rac cells to laminin further suggests that Rac1 is one of the molecules that modulate integrin-mediated adhesion in a negative manner. Rac activation by integrin is thought to be mediated by the FAK-p130 Cas-Crk-DOCK180 pathway and the paxillin-GIT-PIX pathway (Legate et al., 2009). Cell adhesion influences the ability of Rac to activate Pak1 and induces phosphorylation. Also, Rac feeds back to the integrins by interacting with Rho (Parsons et al., 2010), and autophosphorylation of Pak1 triggers degradation of Pak1 (Weisz Hubsman et al., 2007), thereby creating another feedback loop to prevent further Pak1 activation. Interference with these feedback loops by a dominant-negative Rac construct may have enhanced cell adhesion. Because activated Rac was found less frequently in undifferentiated spermatogonia, SSCs in the niche may be less motile and adhere more strongly to the basement membrane (Figure 7).

The results of the present study also showed that Rac is involved in the proliferation of GSCs. Transfection of a dominant-negative Rac construct not only enhanced GSC proliferation, but also induced abnormal expression of cyclin D and p27 CDKI. In particular, decreased *p27* expression suggested that they undergo differentiating divisions, because *p27* KO SSCs preferentially undergo differentiating divisions (Kanatsu-Shinohara et al., 2010). Consistent with this, DN-Rac cells showed reduced colonization in the adult recipients. Although increased adhesion to the niche and promotion of differentiating divisions appear contradictory, adhesion to the niche is necessary but not sufficient for defining SSCs. SSCs must also be able to transmigrate through the BTB; those cells that cannot transmigrate are differentiating cells by definition even if they can attach strongly to the basement membrane.

Because a homing defect alone can impair SSC activity, it was difficult to determine whether reduced colonization of DN-Rac cells was due to impaired self-renewal activity or a migratory defect. This problem was resolved by serial transplantation using pup recipients, which showed that the concentration of SSCs in WT and DN-Rac GSCs in vitro is comparable if the transmigration process is omitted. Moreover, the serial transplantation also showed that the DN-Rac cells proliferate actively also in an in vivo environment and produce significantly more secondary colonies

(B) Macroscopic (left) and histological (right) appearances of the recipient testes. Both types of donor cell produced germ cell colonies. Note the increased size of the recipient testes after transplantation.
(C) Southern blot analysis of deletion efficiency.
(D) Evaluation of spermatogenesis. (Top) Testis weight (n = 9 for the control; n = 10 for mutant). (Bottom) Tubules with spermatogenesis (n = 11). The numbers of tubules counted were 783 (control) and 1,083 (mutant).
(E) RT-PCR analyses of the pup recipient testes, showing stage-specific spermatogenic gene expression. *Hprt* was used as an internal control. No apparent defects in differentiation were observed.
(F) Macroscopic appearance of recipient testes used for serial transplantation. GSCs were transplanted into W pup recipients (primary recipients). Five weeks after transplantation, EGFP-expressing germ cell colonies were dissected out using fine forceps and enzymatically dissociated into single-cell suspensions. Portions of the cells were then transplanted into two testes of a W pup recipient (secondary recipients). Green fluorescence indicates donor cell colonization. Note the increased secondary colonies in the recipients that received DN-Rac cells.
(G) Numbers of colonies in the primary recipients (n = 27 for the control; n = 21 for DN-Rac cells). (H) Multiplication of colony numbers (total regenerated colony number / primary colony number used for serial transplantation) (n = 11).
Scale bar = 500 μm (A); 1 mm (B, left and F); 100 μm (B, right). Stain, hematoxylin and eosin (B). Error bars = SEM. See also Figure S3 and Tables S3 and S5.

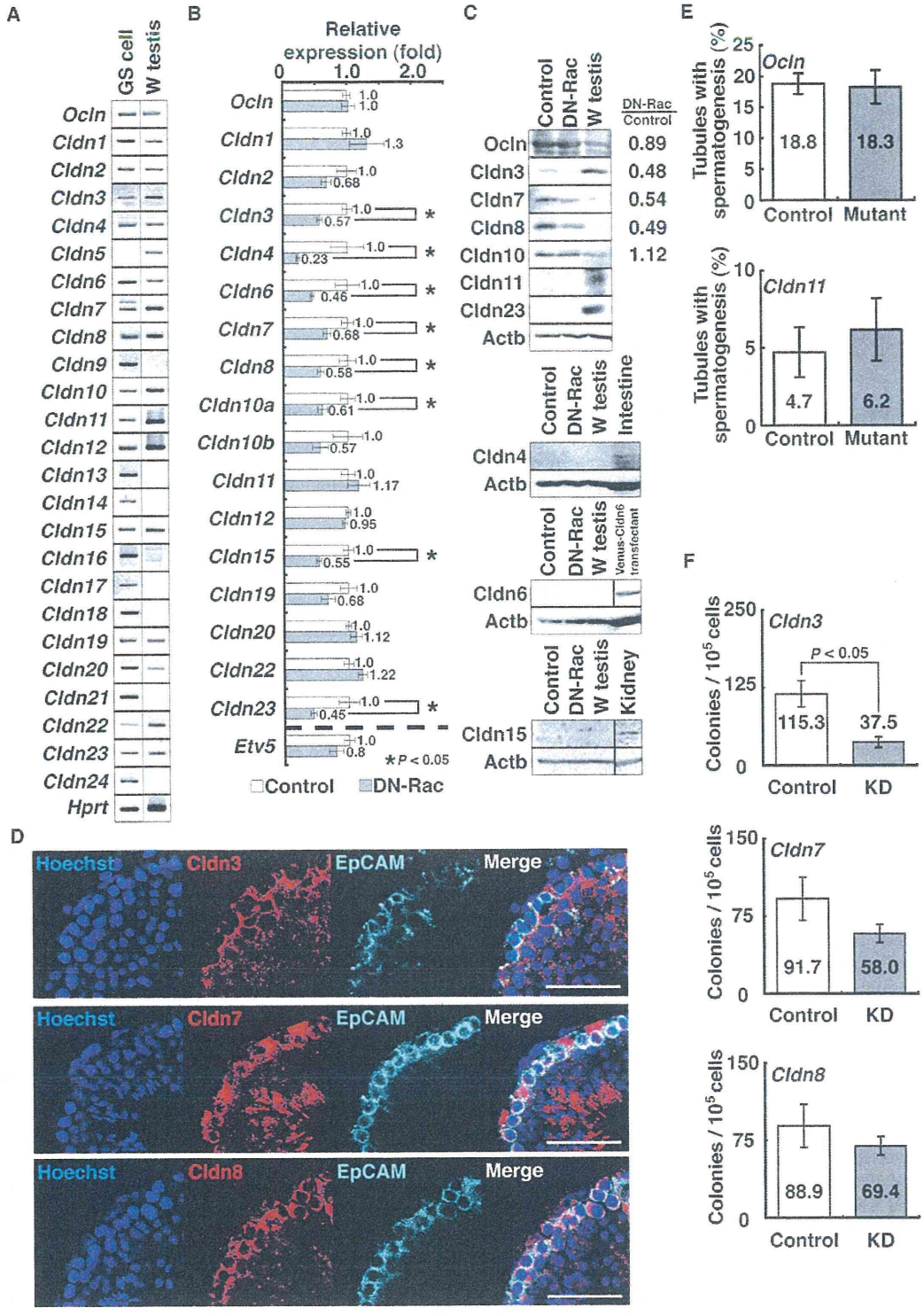

Figure 6. Functional Analyses of Tight Junction-Associated Proteins in SSC Homing

(A) RT-PCR analyses of *Ocln* and *Cldn* genes in GSCs and W testis.

(B) Real-time PCR analyses of commonly expressed genes and *Etv5* in control and Rac-DN cells (n = 6).

(C) Western blot analyses. The numbers along the gel represent the ratios of band intensities. The positive control was made by transfecting *Cldn6* cDNA into 293T cells.

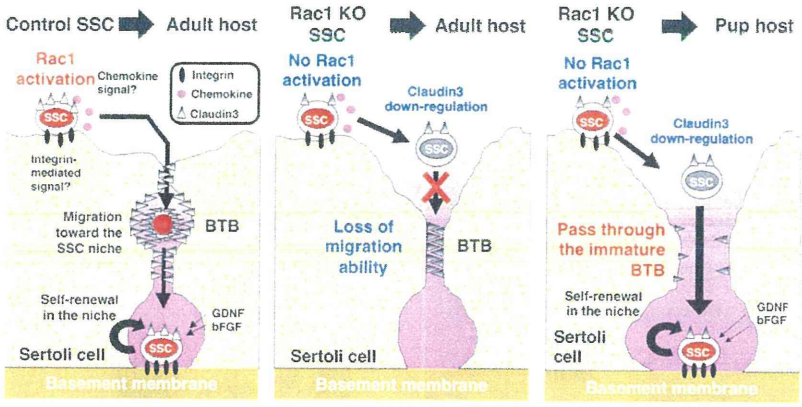

Figure 7. Model for SSC Homing

Rac in SSCs is activated either by chemokines or adhesion to Sertoli cells. SSCs then transmigrate through the BTB by modulating the expression of tight junction-associated proteins before they settle on the basement membrane via β1-integrin. The downregulation of tight junction-associated proteins, including claudin3, by Rac1 inhibition interfered with SSC transmigration. In contrast, SSCs can directly settle on the basement membrane of the seminiferous tubules in the pup testis without a BTB.

than WT cells do. Therefore, Rac inhibition enhances cell proliferation both in vivo and in vitro but does not necessarily compromise self-renewal activity. However, the potentially increased SSC activity, as suggested by pup transplantation, is offset by defective transmigration in the adult seminiferous tubules, which was interpreted as increased differentiating division using adult recipients. Although we currently do not know how Rac inhibition enhanced GSC proliferation, these results suggest that Rac is a critical molecule that controls both SSC transmigration and proliferation.

The next challenge is to identify upstream molecules of Rac signaling, which will be important for understanding the initial phases of SSC homing. At least two possibilities exist: chemokines and attachment to Sertoli cells. Although firm confirmation is lacking, SSCs are considered to be guided by chemokines to the appropriate niche to initiate migration (Nagano et al., 1999). Although GDNF is a chemoattractant factor for some cell types (Paratcha et al., 2006; Tang et al., 1998), no evidence yet exists to indicate that it also attracts SSCs toward the niche. Chemokine signals activate Rac in hematopoietic cells (Cancelas et al., 2006), but whether a similar scenario applies to SSCs is unknown. With regard to the second possibility (i. e., attachment to Sertoli cells), transplanted SSCs attach to the Sertoli cell surface before migrating toward the BTB (Russell et al., 1996). In leukocyte transmigration, integrins on leukocytes are activated by attachment to the endothelium and subsequently participate in firm arrest on the endothelium before transmigration (Worthylake and Burridge, 2001). Although we found that adhesion to laminin can activate Rac, different types of integrins on SSCs may be involved in Sertoli cell attachment and activate Rac, which then modulates the expression of tight junction proteins for transmigration. Gaining an understanding of the mechanism of Rac activation and its targets in endogenous spermatogonia is another important challenge. This may explain why pup spermatogonia proliferate more actively despite the same level of Rac expression. It also remains to be examined why SSCs with reduced Rac activity can undergo self-renewal division during normal spermatogenesis whereas inhibition of Rac activation promotes differentiation division by serial transplantation. Perhaps Rac expression as well as activation levels may change when SSCs are triggered to increase their number. Future studies are required to address these questions.

Since the development of the germ cell transplantation technique, the mechanism of SSC homing has remained unclear. In addition to the hematopoietic system, the spermatogenic system is the only other self-renewing tissue in which a functional transplantation assay exists for stem cell homing. In addition to β1-integrin, we have now determined that a role exists for Rac1 in regulating transmigration between Sertoli cells, which is the most critical aspect of SSC homing. These molecules operate in distinct steps of homing and also play important roles in HSC homing to the bone marrow niche. In contrast to the complex structure of the bone marrow, the relatively simple structure of the testis is advantageous for understanding the homing process, and Rac may regulate homing of other types of stem cells in a similar manner by regulating the expression of tight junction-associated molecules. Comparison between the two self-renewing systems will reveal the common molecular machinery of stem cell homing and will provide insight into the mechanism and regulation of stem-cell-niche interactions.

EXPERIMENTAL PROCEDURES

KO Animals and Transplantation

The generation of *Cldn11*, *Ocln* KO, and *Rac1* conditional mutant mice was described previously (Glogauer et al., 2003; Kitajiri et al., 2004; Saitou et al., 2000). For *Rac1* mutant experiments, male *Rac1* mutant mice were crossed with R26R female mice (Soriano, 1999) to introduce the LacZ reporter construct for Cre-mediated deletion (both from the Jackson Laboratory, Bar Harbor, ME).

Statistical Analyses

Results are presented as means ± SEM. Data were analyzed using Student's t test.

(D) Cldn3, 7, and 8 expression in 8-week-old mouse testes.
(E) Tubules with spermatogenesis after transplantation of *Ocln* and *Cldn11* KO testis cells (n = 6). The numbers of tubules counted were: 639 (control) and 659 (mutant) for occludin: 735 (control) and 663 (mutant) for claudin11.
(F) Colony count after claudin3, 7, and 8 KD in GSCs (n = 18).
Scale bar = 50 μm (D). Error bars = SEM. See also Figure S4 and Tables S1, S2, S3, and S6.

SUPPLEMENTAL INFORMATION

Supplemental Information for this article includes four figures, six tables, and Supplemental Experimental Procedures and can be found with this article online at doi:10.1016/j.stem.2011.08.011.

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REFERENCES

Brinster, R.L., and Zimmermann, J.W. (1994). Spermatogenesis following male germ-cell transplantation. Proc. Natl. Acad. Sci. USA *91*, 11298–11302.

Burridge, K., and Wennerberg, K. (2004). Rho and Rac take center stage. Cell *116*, 167–179.

Cancelas, J.A., Jansen, M., and Williams, D.A. (2006). The role of chemokine activation of Rac GTPases in hematopoietic stem cell marrow homing, retention, and peripheral mobilization. Exp. Hematol. *34*, 976–985.

Chiarini-Garcia, H., Hornick, J.R., Griswold, M.D., and Russell, L.D. (2001). Distribution of type A spermatogonia in the mouse is not random. Biol. Reprod. *65*, 1179–1185.

de Rooij, D.G., and Russell, L.D. (2000). All you wanted to know about spermatogonia but were afraid to ask. J. Androl. *21*, 776–798.

del Pozo, M.A., Price, L.S., Alderson, N.B., Ren, X.-D., and Schwartz, M.A. (2000). Adhesion to the extracellular matrix regulates the coupling of the small GTPase Rac to its effector PAK. EMBO J. *19*, 2008–2014.

Gao, Y., Dickerson, J.B., Guo, F., Zheng, J., and Zheng, Y. (2004). Rational design and characterization of a Rac GTPase-specific small molecule inhibitor. Proc. Natl. Acad. Sci. USA *101*, 7618–7623.

Glogauer, M., Marchal, C.C., Zhu, F., Worku, A., Clausen, B.E., Foerster, I., Marks, P., Downey, G.P., Dinauer, M., and Kwiatkowski, D.J. (2003). Rac1 deletion in mouse neutrophils has selective effects on neutrophil functions. J. Immunol. *170*, 5652–5657.

Gow, A., Southwood, C.M., Li, J.S., Pariali, M., Riordan, G.P., Brodie, S.E., Danias, J., Bronstein, J.M., Kachar, B., and Lazzarini, R.A. (1999). CNS myelin and sertoli cell tight junction strands are absent in Osp/claudin-11 null mice. Cell *99*, 649–659.

Joyce, D., Bouzahzah, B., Fu, M., Albanese, C., D'Amico, M., Steer, J., Klein, J.U., Lee, R.J., Segall, J.E., Westwick, J.K., et al. (1999). Integration of Rac-dependent regulation of cyclin D1 transcription through a nuclear factor-kappaB-dependent pathway. J. Biol. Chem. *274*, 25245–25249.

Kanatsu-Shinohara, M., Ogonuki, N., Inoue, K., Miki, H., Ogura, A., Toyokuni, S., and Shinohara, T. (2003). Long-term proliferation in culture and germline transmission of mouse male germline stem cells. Biol. Reprod. *69*, 612–616.

Kanatsu-Shinohara, M., Inoue, K., Lee, J., Yoshimoto, M., Ogonuki, N., Miki, H., Baba, S., Kato, T., Kazuki, Y., Toyokuni, S., et al. (2004). Generation of pluripotent stem cells from neonatal mouse testis. Cell *119*, 1001–1012.

Kanatsu-Shinohara, M., Ikawa, M., Takehashi, M., Ogonuki, N., Miki, H., Inoue, K., Kazuki, Y., Lee, J., Toyokuni, S., Oshimura, M., et al. (2006). Production of knockout mice by random or targeted mutagenesis in spermatogonial stem cells. Proc. Natl. Acad. Sci. USA *103*, 8018–8023.

Kanatsu-Shinohara, M., Takehashi, M., Takashima, S., Lee, J., Morimoto, H., Chuma, S., Raducanu, A., Nakatsuji, N., Fässler, R., and Shinohara, T. (2008). Homing of mouse spermatogonial stem cells to germline niche depends on β1-integrin. Cell Stem Cell *3*, 533–542.

Kanatsu-Shinohara, M., Takashima, S., and Shinohara, T. (2010). Transmission distortion by loss of p21 or p27 cyclin-dependent kinase inhibitors following competitive spermatogonial transplantation. Proc. Natl. Acad. Sci. USA *107*, 6210–6215.

Kitajiri, S., Miyamoto, T., Mineharu, A., Sonoda, N., Furuse, K., Hata, M., Sasaki, H., Mori, Y., Kubota, T., Ito, J., et al. (2004). Compartmentalization established by claudin-11-based tight junctions in stria vascularis is required for hearing through generation of endocochlear potential. J. Cell Sci. *117*, 5087–5096.

Knaus, U.G., and Bokoch, G.M. (1998). The p21Rac/Cdc42-activated kinases (PAKs). Int. J. Biochem. Cell Biol. *30*, 857–862.

Lee, J., Kanatsu-Shinohara, M., Morimoto, H., Kazuki, Y., Takashima, S., Oshimura, M., Toyokuni, S., and Shinohara, T. (2009). Genetic reconstruction of mouse spermatogonial stem cell self-renewal in vitro by Ras-cyclin D2 activation. Cell Stem Cell *5*, 76–86.

Legate, K.R., Wickström, S.A., and Fässler, R. (2009). Genetic and cell biological analysis of integrin outside-in signaling. Genes Dev. *23*, 397–418.

Li, L., and Xie, T. (2005). Stem cell niche: structure and function. Annu. Rev. Cell Dev. Biol. *21*, 605–631.

Meistrich, M.L., and van Beek, M.E.A.B. (1993). Spermatogonial stem cells. In Cell and Molecular Biology of the Testis, C. Desjardins and L.L. Ewing, eds. (New York: Oxford University Press), pp. 266–295.

Meng, J., Holdcraft, R.W., Shima, J.E., Griswold, M.D., and Braun, R.E. (2005). Androgens regulate the permeability of the blood-testis barrier. Proc. Natl. Acad. Sci. USA *102*, 16696–16700.

Morrow, C.M.K., Tyagi, G., Simon, L., Carnes, K., Murphy, K.M., Cooke, P.S., Hofmann, M.-C.C., and Hess, R.A. (2009). Claudin 5 expression in mouse seminiferous epithelium is dependent upon the transcription factor ets variant 5 and contributes to blood-testis barrier function. Biol. Reprod. *81*, 871–879.

Mruk, D.D., and Cheng, C.Y. (2004). Sertoli-Sertoli and Sertoli-germ cell interactions and their significance in germ cell movement in the seminiferous epithelium during spermatogenesis. Endocr. Rev. *25*, 747–806.

Nagano, M.C. (2003). Homing efficiency and proliferation kinetics of male germ line stem cells following transplantation in mice. Biol. Reprod. *69*, 701–707.

Nagano, M., Avarbock, M.R., and Brinster, R.L. (1999). Pattern and kinetics of mouse donor spermatogonial stem cell colonization in recipient testes. Biol. Reprod. *60*, 1429–1436.

Nagano, M., Brinster, C.J., Orwig, K.E., Ryu, B.Y., Avarbock, M.R., and Brinster, R.L. (2001). Transgenic mice produced by retroviral transduction of male germ-line stem cells. Proc. Natl. Acad. Sci. USA *98*, 13090–13095.

Oatley, J.M., and Brinster, R.L. (2008). Regulation of spermatogonial stem cell self-renewal in mammals. Annu. Rev. Cell Dev. Biol. *24*, 263–286.

Ohta, H., Wakayama, T., and Nishimune, Y. (2004). Commitment of fetal male germ cells to spermatogonial stem cells during mouse embryonic development. Biol. Reprod. *70*, 1286–1291.

Paratcha, G., Ibáñez, C.F., and Ledda, F. (2006). GDNF is a chemoattractant factor for neuronal precursor cells in the rostral migratory stream. Mol. Cell. Neurosci. *31*, 505–514.

Parsons, J.T., Horwitz, A.R., and Schwartz, M.A. (2010). Cell adhesion: integrating cytoskeletal dynamics and cellular tension. Nat. Rev. Mol. Cell Biol. *11*, 633–643.

Russell, L. (1977). Movement of spermatocytes from the basal to the adluminal compartment of the rat testis. Am. J. Anat. *148*, 313–328.

Russell, L.D., França, L.R., and Brinster, R.L. (1996). Ultrastructural observations of spermatogenesis in mice resulting from transplantation of mouse spermatogonia. J. Androl. *17*, 603–614.

Saitou, M., Furuse, M., Sasaki, H., Schulzke, J.D., Fromm, M., Takano, H., Noda, T., and Tsukita, S. (2000). Complex phenotype of mice lacking occludin, a component of tight junction strands. Mol. Biol. Cell *11*, 4131–4142.

Shinohara, T., Orwig, K.E., Avarbock, M.R., and Brinster, R.L. (2001). Remodeling of the postnatal mouse testis is accompanied by dramatic changes in stem cell number and niche accessibility. Proc. Natl. Acad. Sci. USA *98*, 6186–6191.

Soriano, P. (1999). Generalized lacZ expression with the ROSA26 Cre reporter strain. Nat. Genet. *21*, 70–71.

Tang, M.J., Worley, D., Sanicola, M., and Dressler, G.R. (1998). The RET-glial cell-derived neurotrophic factor (GDNF) pathway stimulates migration and chemoattraction of epithelial cells. J. Cell Biol. *142*, 1337–1345.

Weisz Hubsman, M., Volinsky, N., Manser, E., Yablonski, D., and Aronheim, A. (2007). Autophosphorylation-dependent degradation of Pak1, triggered by the Rho-family GTPase, Chp. Biochem. J. *404*, 487–497.

Worthylake, R.A., and Burridge, K. (2001). Leukocyte transendothelial migration: orchestrating the underlying molecular machinery. Curr. Opin. Cell Biol. *13*, 569–577.

Yoshida, S. (2010). Stem cells in mammalian spermatogenesis. Dev. Growth Differ. *52*, 311–317.