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Report

Conversion of Quiescent Niche Cells to Somatic Stem Cells Causes Ectopic Niche Formation in the *Drosophila* Testis

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SUMMARY

Adult stem cells reside in specialized regulatory microenvironments, or niches, where local signals ensure stem cell maintenance. The *Drosophila* testis contains a well-characterized niche wherein signals from postmitotic hub cells promote maintenance of adjacent germline stem cells and somatic cyst stem cells (CySCs). Hub cells were considered to be terminally differentiated; here, we show that they can give rise to CySCs. Genetic ablation of CySCs triggers hub cells to transiently exit quiescence, delaminate from the hub, and convert into functional CySCs. Ectopic Cyclin D-Cdk4 expression in hub cells is also sufficient to trigger their conversion into CySCs. In both cases, this conversion causes the formation of multiple ectopic niches over time. Therefore, our work provides a model for understanding how oncogenic mutations in quiescent niche cells could promote loss of quiescence, changes in cell fate, and aberrant niche expansion.

INTRODUCTION

Niches are specialized microenvironments that regulate tissue-specific stem cells via local signals. Understanding how niches ensure stem cell renewal, particularly in response to age or tissue damage, remains challenging due to the complexity of most niches (Hsu and Fuchs, 2012). The cells within a tumor that sustain long-term propagation of cancer (cancer stem cells) are also thought to reside in niches, but the biogenesis of cancer stem cells and their niches is poorly understood (Visvader and Lindeman, 2012).

One of the best characterized niches is found in the *Drosophila* testis apex, where a cluster of quiescent somatic cells called the hub creates a niche that maintains adjacent germline stem cells (GSCs) and somatic cyst stem cells (CySCs) (Figure 1A) (de Cuevas and Matunis, 2011). GSCs and CySCs divide asymmetrically, producing new stem cells (self-renewal) and daughters that are displaced from the hub and differentiate. GSCs give rise to gonialblasts, which undergo four rounds of mitosis with incomplete cytokinesis to form clusters of spermatogonia, whereas CySCs give rise to postmitotic cyst cells, which envelop dividing germ cells and sustain their development. GSCs lost through damage or aging are typically replaced by remaining GSCs but can also arise from dedifferentiation of spermatogonia (Brawley and Matunis, 2004; Cheng et al., 2008; Sheng et al., 2009). How lost CySCs are replaced, however, is not understood.

Here, we show that quiescent hub cells can convert into functional CySCs after complete genetic ablation of CySCs or forced expression of Cyclin D and Cyclin-dependent kinase 4 (Cdk4) in the hub, but not in response to partial loss of CySCs. As a consequence of this conversion, multiple ectopic niches can form in the testis over time. Our results suggest that hub cells can give rise to CySCs and provide a simple model for understanding how lost stem cell populations can be replaced by activation of quiescent niche cells.

RESULTS

CySCs in the Adult Testis Can Be Genetically Ablated

To ask if CySCs can be restored after ablation, we first established conditions to genetically ablate all CySCs in the adult testis. We used the GAL4-UAS system to conditionally express the proapoptotic gene *grim* in all CySCs and cyst cells associated with spermatogonia (early cyst cells) (Figure 1B). At the permissive temperature of 18°C, testes were phenotypically wild-type (Figures 1C and 1D); by contrast, after shifting flies to the restrictive temperature of 31°C for 2 days, 100% of testes completely lacked CySCs and early cyst cells, as shown by immunostaining for the CySC and early cyst cell nuclear marker Traffic jam (Tj; Figure 1E) (Lasko and Ashburner, 1990; Li et al., 2003) and the CySC nuclear marker Zinc finger homeodomain 1 (Zfh1; Figure 1F; Table S1a) (Leatherman and DiNardo, 2008). As expected, cells that did not express ectopic *grim* were still present in all testes after 2 days, including hub cells, outlined by the adhesion protein Cadherin-N (CadN; Figure 1F) (Sinden et al., 2012); late cyst cells, which surround spermatocytes and express the nuclear marker Eyes absent (Eya; Figure S1a) (Fabrizio et al., 2003); and germ cells (GSCs, spermatogonia, and spermatocytes), which express the germline marker Vasa (Figure S1b). Furthermore, every cell that remained outside the hub expressed either germ cell or late cyst cell markers; therefore, CySCs and early cyst cells are being lost, not simply turning off markers. Consistent with this finding,

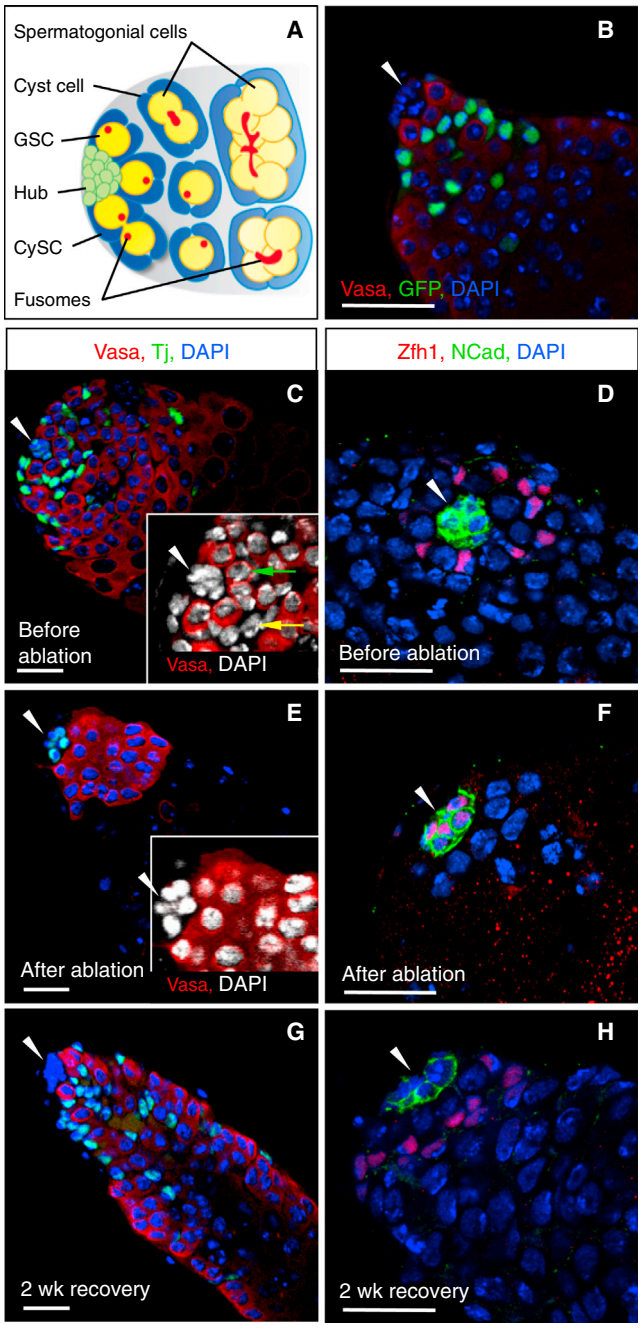

Figure 1. Recovery of Functional CySCs after Complete Genetic Ablation of CySCs and Early Cyst Cells in the Adult Testis

(A) The *Drosophila* testis apex. About ten germline stem cells (GSCs, yellow) adhere to the hub (green), a cluster of 15–20 quiescent somatic cells. GSCs divide asymmetrically, producing new stem cells (self-renewal) and daughters that are displaced from the hub and differentiate. Differentiating daughters form clusters of spermatogonia, which differentiate into spermatocytes. Somatic cyst stem cells (CySCs, blue) flank each GSC and adhere to the hub via thin cytoplasmic extensions. CySCs self-renew and produce cyst cells, which envelop differentiating germ cells. The fusome (red) is round in GSCs and gonialblasts and elongated or branched in spermatogonial clusters.

(B–H) Single confocal sections through the testis apex immunostained with antibodies as indicated. Nuclei are counterstained with 4′,6-diamidino-2-phenylindole (DAPI, blue). The hub is indicated (arrowhead). (B) Testis expressing GFP under the control of the *c587-Gal4* driver immunostained with anti-GFP (green; CySCs and early cyst cells associated with spermatogonia) and anti-Vasa (red; germ cells). (C–H) Testes from *c587-Gal4; UAS-grim/+; tub-Gal80^{ts}/+* flies (referred to as *c587-Gal4>grim* hereafter) immunostained with (C, E, and G) anti-Vasa (red; germ cells) and anti-Tj (green; hub cells, CySCs, and early cyst cells) or (D, F, and H) anti-Zfh1 (red; hub cells, CySCs and their immediate daughters) and anti-CadN (green; hub cells). (C and D) Before Grim expression (18°C), testes appear wild-type: Tj-positive and Zfh1-positive cells are found within all testes (n = 249 and 64 testes, respectively). (C, inset) A single CySC (yellow arrow) and GSC (green arrow) are indicated. (E and F) After 2 days of Grim expression (31°C), all CySCs and early cyst cells are completely ablated. No Tj-positive or Zfh1-positive cells are found outside the hub (n = 728 and 85 testes, respectively). All remaining cells outside the hub are DAPI-positive/Vasa-positive; no Vasa-negative cells are observed (E, inset). Incidentally, at 31°C, hub cells express higher levels of Tj and Zfh1; this is due to temperature, not CySC ablation, because it also occurs in control testes at 31°C (not shown). (G and H) After ablation (2 days at 31°C) and 2 weeks of recovery (18°C), testes can regain CySCs and early cyst cells. Scale bars, 20 μm. See also Figure S1 and Table S1.

ectopic *grim* expression in the CySC lineage induced apoptosis in CySCs but not in hub cells or GSCs (Figure S1c; Table S1b). Because CySC-to-hub cell conversion was reported to occur with aging (Voog et al., 2008), but contradictory results were also reported (DiNardo et al., 2011), we asked if any CySCs escape ablation by becoming hub cells. First, we fed flies the thymidine analog ethynyl deoxyuridine (EdU) to label cells undergoing DNA replication. We dissected half the flies after 72 hr of labeling and found that EdU was incorporated into their germ cells and CySC lineage cells but was not detected in the hub (Figure S1d). In 75% of testes (n = 18/24), 100% of CySCs were labeled with EdU; in the remaining testes (n = 6/24), all but one or two CySCs were labeled. We then shifted the remaining flies to 31°C for 2 days to ablate CySCs. EdU was not detected in the hub in any testis after ablation, although it was still detected in germ cells (n = 25 testes; Figure S1d). Therefore, CySCs marked by EdU did not enter the hub upon CySC ablation. We conclude that ectopic expression of *grim* in CySCs and early cyst cells for 2 days autonomously induces cell death of all CySCs and early cyst cells; we refer to these conditions as "CySC ablation."

CySCs Are Regenerated after Ablation

To determine the effects of CySC ablation on the remaining cells in the testis, we returned flies lacking CySCs to the permissive temperature (18°C) and allowed them to recover for 2 weeks. As expected from previous findings (Lim and Fuller, 2012), 35% of testes (n = 178/506) were "empty" except for the hub, or the hub and early germ cells (not shown). Unexpectedly, 65% of testes (n = 328/506) appeared strikingly similar to wild-type testes: they retained a hub and germ cells but also regained a large population of Tj-positive somatic cells intermingled with germ cells (Figure 1G). Staining for Zfh1 indicated that CySCs had returned (Figure 1H). To determine if the new somatic cells were functional, we assayed for the presence of spermatocytes, which cannot form in the absence of cyst cell-derived signals (Lim and Fuller, 2012; Zoller and Schulz, 2012). Although spermatocytes remained immediately after CySC ablation, they were gone from most testes by 1 week of recovery, as expected after a lapse in cyst cell production. By 2 weeks of recovery, however,