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Rap Signaling Regulates Stem Cell Anchoring in *Drosophila* Testis

Wang H, Singh SR, Zheng Z, Oh SW, Chen X, Edwards K, and Hou SX. Rap-GEF signaling controls stem cell anchoring to their niche through regulating DE-cadherin–mediated cell adhesion in the *Drosophila* testis. *Dev Cell* 10: 117–26, 2006.

Stem cells can either self-renew or differentiate into short-lived cell types. Cancer cells also possess the potential for self-renewal; tumors may originate from a few transformed cancer stem cells (Reya T et al. *Nature* 414: 105–11, 2001). Understanding the molecular mechanisms that control stem cell self-renewal versus differentiation is crucial to the use of stem cells in regenerative medicine and the development of effective anticancer therapies. Accumulated evidence suggests that stem cells are controlled by particular microenvironments known as niches (Spradling A et al. *Nature* 414: 98–104, 2001; Fuchs E et al. *Cell* 116: 769–78, 2004). A niche is a subset of neighboring stromal cells and extracellular substrates. The stromal cells often secrete growth factors to regulate stem cell behavior.

The *Drosophila* testis provides an excellent *in vivo* system to study stem cells and niches at the cellular and molecular levels (Fuller MT. *Semin Cell Dev Biol* 9: 433–44, 1998; Yamashita YM et al. *J Cell Sci* 118: 665–72, 2005). At the tip of the *Drosophila* testis (the apex) is a germinal proliferation center, which contains the germline and somatic stem cells that maintain spermatogenesis. Each adult male fly testis has five to nine germline stem cells (GSCs), each encysted by two somatic stem cells (SSCs, also called cyst progenitor cells). Both GSCs and SSCs attach to a group of 12 nondividing somatic cells called the hub (Hardy RW et al. *J Ultrastruct Res* 69: 180–90, 1979; Gonczy P and DiNardo S. *Development* 122: 2437–47, 1996). The hub defines the stem cell niche by expressing the growth factor Unpaired (Upd), which activates the JAK/STAT pathway in GSCs to regulate the stem cell self-renewal process (Kiger AA et al. *Science* 294: 2542–5, 2001; Tulina N and Matunis E. *Science* 294: 2546–9, 2001). Meanwhile, a member of the transforming growth factor-β (TGF-β) family, glass bottom boat (Gbb), is also expressed in the hub and plays a part in regulating GSC self-renewal by activating its corresponding signal transduction pathway in the GSCs (Kawase E et al. *Development* 131: 1365–75, 2004). Because Upd and Gbb are expressed in the hub, they have very limited ability to diffuse; therefore, the GSCs must first be anchored to the hub to receive the signals and maintain their stem cell identity.

The cell adhesion molecules E-cadherin and β-catenin (named Armadillo [Arm] in *Drosophila*) are concentrated at the hub-GSC interface and may anchor the stem cells to the niche (Yamashita YM et al. *Science* 301: 1547–50, 2003). However, how the adherens junctions are specifically formed at the hub-GSC interface is not clear. We recently identified in a genetic screen a *Drosophila* small GTPase Rap guanine nucleotide exchange factor (Gef26) as a major regulator of the anchoring of stem cells to their niche. The Gef 26 protein has a PDZ domain, a Ras-binding domain, a cAMP/cGMP-binding domain, and a Rap-binding domain. Mutations of Gef26 cause loss of both GSCs and SSCs in the fly testis. We demonstrated that the Rap-Gef (Gef26)/Rap signaling controls stem cell anchoring to the niche through regulation of E-cadherin–mediated cell adhesion. The *Gef26* mutation specifically impairs adherens junctions at the hub–stem cell interface, which results in the stem cells "drifting away" from the niche and losing stem cell identity (Figure 1). Thus, the Rap signaling/E-cadherin pathway may represent one mechanism that regulates polarized niche formation and stem cell anchoring (Wang H et al. *Dev Cell* 10: 117–26, 2006).

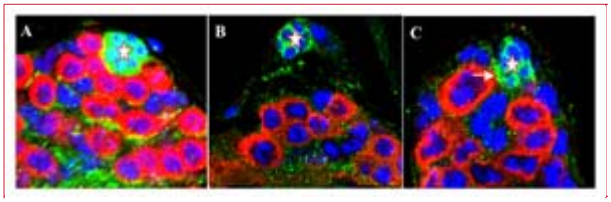

Figure 1. Germline stem cells (GSCs) "drift away" from the niche in *Gef26* mutant testes because of impaired adherens junctions. The Wild-type *(A)*, *Gef26*^{4} *(B)*, and *Gef26*^{6} *(C)* testes of adult flies were immunostained with anti–E-cadherin (green), anti-Vasa (red), and DAPI (blue). In *(A)*, all GSCs are anchored to the hub (star) through E-cadherin–positive adherens junctions. In *(B)*, all Vasa-positive germ cells drifted away from the hub (star). In *(C)*, only one GSC is still attached to the hub (star) through E-cadherin–positive adherens junctions (arrow).

Rap1 was first identified as a gene that can reverse the transformed phenotype of fibroblasts by one of the mutated Ras genes, K-*ras* (Kitayama H et al. *Cell* 56: 77–84, 1989). Rap1 belongs to the Ras family of small GTP-binding proteins. Its apparent tumor suppressor properties were initially proposed to antagonize the activity of Ras by competing for a common target (or regulatory protein). However, recent studies have suggested that Rap1 may actually regulate adherens junctions. In a recent study, a Rap1 GTPase activator, Dock4, was identified as a tumor suppressor (Yajnik V et al. *Cell* 112: 673–84, 2003). Dock4 specifically activates Rap1 and regulates the formation of adherens junctions. Our recent results show that the Rap-Gef (Gef26)/Rap signaling controls the anchoring of stem cells to their niche through regulation of E-cadherin–mediated cell adhesion in the *Drosophila* testis. The mammalian homolog of Gef26 may regulate cancer stem cell anchoring and function as a tumor suppressor. We are in the process of knocking out the homologous *Gef26* gene in mice (*RapGef2*) and searching for an association between RAPGEF2 and human diseases. Thus, the powerful genetic manipulations available in *Drosophila* in combination with the mouse knockout studies may make this an ideal system to study cancer stem cells and cancers.

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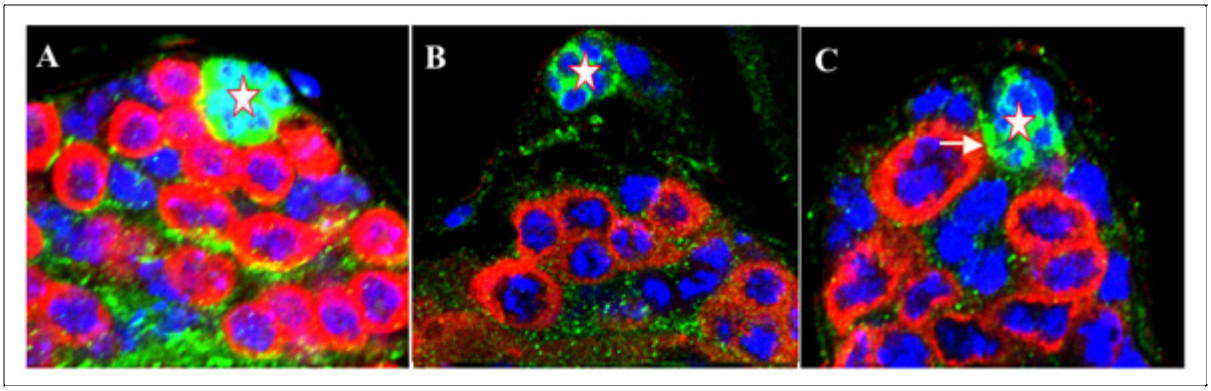

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