**Drosophila Perlecan Regulates Intestinal Stem Cell Activity via Cell-Matrix Attachment**

Jia You,1,2 Yan Zhang,3 Zhouhua Li,3 Zhefeng Lou,4 Longjin Jin,4 and Xinhua Lin1,2,3,*

1Division of Developmental Biology, Cincinnati Children’s Hospital Medical Center, Cincinnati, OH 45229, USA
2The Graduate Program in Molecular and Developmental Biology, University of Cincinnati College of Medicine, Cincinnati, OH 45229, USA
3State Key Laboratory of Biomembrane and Membrane Biotechnology, Institute of Zoology, Chinese Academy of Science, Beijing 100101, China
4Zhejiang Provincial Key Laboratory for Medical Genetics, School of Life Sciences, School of Laboratory Medicine, Wenzhou Medical University, Wenzhou 325035, China

*Correspondence: xinhua.lin@cchmc.org
http://dx.doi.org/10.1016/j.stemcr.2014.04.007
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/3.0/).

**SUMMARY**

Stem cells require specialized local microenvironments, termed niches, for normal retention, proliferation, and multipotency. Niches are composed of cells together with their associated extracellular matrix (ECM). Currently, the roles of ECM in regulating niche functions are poorly understood. Here, we demonstrate that Perlecan (Pcan), a highly conserved ECM component, controls intestinal stem cell (ISC) activities and ISC-ECM attachment in *Drosophila* adult posterior midgut. Loss of Pcan from ISCs, but not other surrounding cells, causes ISCs to detach from underlying ECM, lose their identity, and fail to proliferate. These defects are not a result of a loss of epidermal growth factor receptor (EGFR) or Janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling activity but partially depend on integrin signaling activity. We propose that Pcan secreted by ISCs confers niche properties to the adjacent ECM that is required for ISC maintenance of stem cell identity, activity, and anchorage to the niche.

**INTRODUCTION**

Stem cell niches are specialized local microenvironments that are able to house and maintain stem cells (Fuchs et al., 2004; Morrison and Spradling, 2008). Previous studies have shown that stem cell niches are composed of supporting cells and their associated extracellular matrix (ECM) (Chen et al., 2013; Jones and Wagers, 2008; Lander et al., 2012). Supporting cells can regulate stem cells by secreting diffusible factors or through adheren junctions (Chen et al., 2013; Jones and Wagers, 2008; Xie and Spradling, 2000). However, roles of ECM in niches are less understood.

The ECM is thought to be an important component of niche because in many cases, stem cells directly contact the ECM (Chiarini-Garcia et al., 2003; Collins et al., 2005; Kanatsu-Shinohara et al., 2008; Kuang et al., 2008; Shen et al., 2008; Watt, 2002). Particularly, some stem cells, including mouse skeleton muscle satellite cells, mouse skin basal keratinocytes, and *Drosophila* intestinal stem cells (ISCs), are not associated with any specialized supporting cells but are located adjacent to the basement membrane (Kuang et al., 2008; Micchelli and Perrimon, 2006; Ohlstein and Spradling, 2006; Watt, 2002). So far, niches for these stem cells are difficult to define. One possibility is that stem cell niches are formed around stem cells and are established by stem cells through stem cell-ECM interaction.

*Drosophila* adult posterior midgut is an ideal system to investigate the roles of ISC-ECM interaction. In this system, ISCs are individual small cells that reside on the basement membrane and are surrounded by mature epithelial cells (Micchelli and Perrimon, 2006; Ohlstein and Spradling, 2006). Under the basement membrane is a layer of muscle cells. In normal homeostasis, ISCs produce new mature epithelial cells, including abundant big enterocytes (ECs) and rare small enteroendocrine (ee) cells, to replenish the gut every 1–2 weeks. In response to the damage of gut epithelium caused by ingestion of cytotoxic agent dextran sodium sulfate (DSS), ISCs overproliferate and thus help gut epithelium to regenerate itself (Amcheslavsky et al., 2009; Lucchetta and Ohlstein, 2012). Although the functions of signaling pathways in ISC regulation have been intensively studied, what is an ISC niche and how it is established are not understood.

Perlecan (Pcan) is a highly conserved basement membrane-specific heparan sulfate proteoglycan (HSPG) and is composed of a core protein with heparan sulfate chains attached (Cohen et al., 1993; Lin, 2004). Pcan is deposited to the ECM by producing cells and crosslinks with many ECM components (Friedrich et al., 2000). It is encoded by *HSPG2* in mammals and *trol* in *Drosophila* (Kallunki et al., 1991; Voigt et al., 2002). Here, we demonstrated that Pcan plays critical roles in the regulation of ISC activity by mediating stem cell-ECM attachment. Our results suggest that ISC secretes Pcan to form an activated ECM and, therefore, establish a niche for itself.
RESULTS

Loss of Pcan Leads to Loss of ISC Activity and Identity

To determine the roles of Pcan in ISC regulation, we generated positively marked wild-type homozygous mutant (trol+/+) clones with a trolnull allele using the MARCM (mosaic analysis with a repressible cell marker) technique (Lee and Luo, 1999). Ten days after clone induction (ACI), a GFP-marked wild-type (WT) ISC was able to generate 10–15 GFP-labeled cells to form a clone (Figure 1A). In contrast, trolnull clones usually only contained one to two cells (Figure 1A, arrowhead), suggesting that trolnull ISCs lost their ability to produce new cells.
A study suggested that in homozygous the GAL4/upstream activating sequence (UAS) system. 20 transcripts, it is difficult to ectopically express it using expression of control (Figures 1 E and 1F). In rescue experiment, GFP RNAi, the number of progenitor cells and ISCs was knocked down in progenitor cells by the expression of hypomorphic alleles to generate clones. Both trolG0271/G0271 and trolG0021/G0023 clones contained only one to two cells, which were similar to trol−/− clones (Figures S1A–S1C available online). In addition, when trol was knocked down in progenitor cells (ISCs and enteroblast [EB] cells) by the expression of trol RNAi driven by esg-gal4, the number of progenitor cells and ISCs was obviously reduced (Figures S1D and S1E). Together, these results demonstrated that loss of Pcan led to loss of stem cell identity and activity.

Because gut epithelium is able to regenerate when it is damaged by DSS (Amcheslavsky et al., 2009; Lucchetta and Ohlstein, 2012), we examined the roles of Pcan in regeneration. Ten days ACI, WT clones in DSS-treated flies were obviously larger than clones in sucrose-treated control flies (Figures 1B and 1C). However, trol−/− clones in DSS-treated flies contained only one to two cells and were significantly smaller than WT clones in DSS-treated flies (Figures 1B and 1C). These results demonstrated that Pcan is required for ISC activities during gut regeneration.

We also performed rescue experiments to show that expression of trol can rescue loss of ISC identity and activity. Because trol is larger than 100 kb and has more than 20 transcripts, it is difficult to ectopically express it using the GAL4/upstream activating sequence (UAS) system. A study suggested that in homoygous trolGFP (trolGFP/GFP) flies, expression of GFP RNAi could knock down trolGFP and therefore deplete normal Pcan function (Pastor-Pareja and Xu, 2011). Here, in trolGFP/GFP flies, when trolGFP was knocked down in progenitor cells by the expression of GFP RNAi, the number of progenitor cells and ISCs was obviously reduced (Figures 1E and 1F). In rescue experiments, with the involvement of a copy of WT trol whose expression is not affected by GFP RNAi, the number of progenitor cells and ISCs was increased and had no significant difference from that in control (Figures 1E and 1F). These results demonstrated that Pcan plays critical roles in the regulation of ISC activity and identity.

**Pcan-Deficient ISCs Lost Their Ability to Proliferate**

We further asked whether loss of Pcan led to cell death. We examined this possibility by determining the levels of cleaved caspase-3 (Casp3) using fluorescence immunostaining. In normal conditions, the levels of cleaved Casp3 were very low, and no cleaved Casp3 could be detected in trol−/− clones (Figures S2A and S2B). When guts were damaged by DSS treatment, the overall levels of cleaved Casp3 were increased. In trol−/− clones, the levels of cleaved Casp3 were not higher than surrounding cells (Figures S2C and S2D). These data argue that loss of Pcan did not lead to cell death.

Next, we determined whether trol−/− ISCs lose their activity due to loss of proliferation. We examined the levels of phosphorylated histone H3 (pH3) by fluorescence immunostaining in guts with WT or trol−/− clones. Usually, around 5% of WT clones contained a pH3+ cell(s), whereas less than 1% of trol−/− clones did (Figures 2A and 2B). We also performed a 5-ethynyl-2′-deoxyuridine (EdU) incorporation assay and found that, compared to WT clones, trol−/− clones contained significantly less EdU+ cells, suggesting that trol−/− ISCs lost their proliferation ability (Figures 2C and 2D).

Janus kinase/signal transducer and activator of transcription (JAK/STAT) and epidermal growth factor receptor (EGFR) signaling are two major signaling pathways that control the ISC proliferation in both normal homeostasis and epithelium regeneration (Beebe et al., 2010; Jiang et al., 2009, 2011; Liu et al., 2010; Ren et al., 2010; Xu et al., 2011). Also, HSPGs are able to interact with many ligand proteins to regulate signaling activities (Hayashi et al., 2012; Lin, 2004; Zhang et al., 2013). Therefore, one possibility is that Pcan may control JAK/STAT and EGFR signaling activities to regulate ISC proliferation. To test this possibility, we examined the activities of JAK/STAT signaling with 10XSTAT92E-GFP and pSTAT staining, and EGFR signaling with dpERK staining in WT and trol−/− clones (Bach et al., 2007; Gabay et al., 1997; Zhang et al., 2013). From 3 to 15 days ACI, the percentage of 10XSTAT92E-GFP+ cells in trol−/− clones was not obviously reduced compared to that in WT clones (Figures 2E and 2G). The percentage of pSTAT+ cells in trol−/− clones showed no significant difference from that in WT clones (Figures 2F and 2H). In addition, the percentage of dpERK+ cells in trol−/− clones was not significantly different from that in WT clones (Figures 2I and 2J). Based on these findings, we conclude that the loss of proliferation in trol−/− ISCs is not the consequence of loss of JAK/STAT or EGFR signaling activity.

**Pcan Regulates ISCs in a Cell-Autonomous Manner**

Because Pcan is a major component of the ECM, we speculated that Pcan may act to maintain the integrity of the...
ECM and thus help ISCs to maintain their identity and activity via interaction with the ECM. In the posterior midgut, high levels of Pcan were detected in the basement membrane, which is located on top of muscle cells (Figure 3A), and low levels of Pcan can be detected around muscle cells.

knockdown in muscle cells by control RNAi driven by 24B-gal4 or mef2-gal4 led to the depletion of Pcan in the muscle layer and reduction of Pcan in the basement membrane (Figures 3 B and 3C). When control was knocked down in ECs using NP1-gal4, the levels of Pcan in the basement membrane were also obviously reduced (Figure 3 D). However, in all cases, the number of ISCs was not reduced (Figures S3A–S3C and S3F), suggesting that the overall levels of Pcan in the basement membrane are not essential for the maintenance of ISC identity and activity.

Importantly, we observed a significantly reduced number of ISCs when control was knocked down in ISCs (Figures S3E and S3F). These results are consistent with the data showing that trol−/− clones only contained one to two cells and suggest that Pcan secreted by surrounding WT cells cannot rescue the deficiency in trol−/− ISCs. We also examined the levels of Pcan in the basement membrane and did not find obvious differences between the levels of Pcan under WT ISCs and that under trol−/− ISCs (Figures 3E and 3F). Consistent with this result, when trol was knocked down in ISCs or EB cells, the levels of Pcan in the basement membrane were not changed (Figures S3G–S3J). Together, these results demonstrate that Pcan regulates ISCs in a cell-autonomous manner.

Pcan Is Required for ISC-ECM Attachment

How does Pcan regulate ISC activity cell autonomously? One possibility is that Pcan may confer its producing ISCs to the local microenvironment required to maintain ISC activities. To test this possibility, we examined trol−/− ISC behavior and interaction with the basement membrane.

Figure 2. trol−/− ISCs Lost Their Proliferation, but Not JAK/STAT or EGFR Signaling Activities

(A and B) pH3 staining (white) showed mitosis cells in clone.
(C and D) EdU incorporation (red) showed proliferating cells in clone.
(E–H) 10XSTAT92E-GFP (green) and pSTAT staining (white) showed that JAK/STAT signaling remained activated in trol−/− clones. p values are shown on top of bars in (G).
(I and J) dpERK staining (white) showed that EGFR signaling remained activated in trol−/− cells.
Scale bars, 10 μm. Dotted lines outline clones. ***p < 0.001; n.s., nonsignificant difference. Error bars show SEs. In (B), (D), (G), (H), and (J), more than 15 flies were analyzed for each group. See also Figure S2.
As shown in Figure 3G, we marked WT or trol/C0 ISC with GFP and the basement membrane with Laminin. Ten days ACI, a WT ISC stayed at the basal side of the gut epithelium and tightly attached to the basement membrane (Figure 3G). However, trol/C0 ISC elongated along the apical-basal axis, moved to the apical side, and finally lost their attachment to the basement membrane (Figure 3G). We further examined the polarity of ISCs with immunostaining of adherens junction components Armadillo (Arm; Drosophila β-catenin) and DE-cadherin (E-CAD; Drosophila E-cadherin). Normally, Arm and E-CAD only stayed in the apical and lateral side and were absent from the basal side (Figures 3H and S3K). High levels of Arm and E-CAD can be detected at the interface membrane between the ISCs and their adjacent EB cells (Figure S3L, arrows) (Maeda et al., 2008; Ohlstein and Spradling, 2006). However, in trol/C0 ISCs, Arm mis-localized to the basal side and could be detected all around ISCs (Figure 3H). Similar to Arm, E-CAD also mis-localized to the basal side of ISCs (Figures S3K and S3L). These results demonstrated that trol/C0 ISCs lost their polarity and their attachment to the basement membrane, indicating that Pcan might help create the proper microenvironment for normal ISC attachment and polarity.

Functions of Pcan Are Partially Dependent on Integrin Signaling Pathway

We further examined the mechanism(s) by which Pcan regulates ISC activity and cellular properties (polarity and cell-ECM attachment). Integrin signaling pathway plays important roles in mediating cell-ECM attachment (Ellis and Tanentzapf, 2010; Hynes, 2002). To determine whether integrin signaling activities were impaired in trol/C0 ISCs, we examined the levels of phosphorylated FAK (pFAK) with anti-pFAK immunostaining (Ivankovic-Dikic et al., 2000). In WT ISCs, pFAK usually accumulated at the attachment point (Figure S4A, arrows), whereas in trol/C0 ISCs, such attachment points with pFAK staining cannot be detected (Figure S4A). To further confirm that Pcan is required for normal integrin signaling activity, we generated trol/C0 clones in wing discs and examined the levels of pFAK as well. Levels of pFAK in trol/C0 clones were obviously reduced compared to those in surrounding WT cells (Figure S4B). Therefore, without Pcan, integrin signaling activity was impaired.

Next, we examined the roles of integrin signaling in the regulation of ISC activity. In Drosophila, mew encodes for the α1-integrin receptor, and mys encodes for the β-integrin
receptor (Brown, 2000). High levels of both Mew and Mys were detected around progenitor cells (Figures S4C and S4D). Similar to trol−/− clones, mys−/− clones were significantly smaller than WT clones (Figures 4A and 4B). Moreover, cells in mys−/− clones were not positive for Dl, Pros, or Pdm1 staining, suggesting that mys−/− ISCs lost their activity and identity. Together with the data showing that integrin signaling activity was reduced in trol−/− clones (Figures S4A and S4B), our data suggest that trol−/− ISCs may lose their identity and activity due to loss of integrin signaling activity.

Figure 4. Functions of Pcan Partially Depend on Integrin Signaling Activity
(A and B) A WT clone and a mys−/− clone 10 days ACI (A). Quantification of the number of total cells per clone from 3 to 20 days ACI is shown (B).
(C and D) The mys rescue clone 5 days ACI (C). Quantification of the number of total cells per clone is shown (D).
(E) The percentage of Dl+ cells and Pros+ cells in WT, trol−/−, and mys rescue clones.
(F) A mys−/− ISC (marked by GFP; green) was tightly attached to the basement membrane (showed by Laminin staining; red).
(G) A trol−/− ISC with mys expression (green) was detached from the basement membrane.
Scale bars, 20 μm (A and C) and 5 μm (F and G). Dotted lines outline clones. **p < 0.01; ***p < 0.001; n.s., nonsignificant difference. Error bars show SEs. In (B), (D), and (E), more than 15 flies were analyzed for each group. See also Figure S4.
DISCUSSION

Our data presented here demonstrate that the ECM protein Pcan plays critical roles in the regulation of ISC activity by mediating ISC-ECM attachment. Because Pcan is a secreted protein that is distributed in ECM (Friedrich et al., 2000), it is surprising to find that Pcan regulates ISCs in a cell-autonomous manner. How could Pcan have cell-autonomous functions? We observed that trol−/− ISCs detached from the basement membrane and lost their polarity, suggesting that ISC-ECM interaction was disrupted. This defect is independent of the integrin signaling pathway because mys−/− ISCs did not show this detachment phenotype. Consistent with this view, we show that the activation of integrin signaling could not rescue the detachment phenotype in trol−/− ISCs. On the basis of these data, we propose that Pcan forms a complex with a cell surface and transmembrane protein(s) before being secreted. Once this complex is presented to the cell surface, Pcan crosslinks to ECM components and forms an activated ECM around the ISC. This activated ECM is required for the ISC anchorage to the basement membrane and, thus, helps to establish the ISC niche. Previous studies show that Pcan binds to Dystroglycan (Dg), a widely expressed ECM receptor, to mediate ECM-cytoskeleton linkage in Drosophila follicle cells (Schneider et al., 2006). Therefore, Dg might be a good candidate for binding Pcan to control the ISC-ECM attachment.

Importantly, the detached trol−/− ISCs lost their ability to proliferate, suggesting that the ISC-ECM attachment is important for the regulation of ISC proliferation. Previous studies show that ISC-ECM attachment is required for maintaining stem cells in the correct position to receive diffusible factors such as bone morphogenetic protein, EGF, and cytokines to maintain the stem cell activity (Ellis and Tanentzapf, 2010; Marthiens et al., 2010; Morrison and Spradling, 2008). However, in detached trol−/− ISCs, JAK/STAT and EGFR signaling pathways remained activated, demonstrating that the ISC does not need to be anchored to the basement membrane to receive JAK/STAT or EGFR signaling. Also, even though JAK/STAT and EGFR signaling pathways remained activated, the detached trol−/− ISCs still cannot proliferate, indicating that ISC-ECM attachment regulates ISC proliferation in a manner independent of JAK/STAT and EGFR signaling. Interestingly, we observed that integrin signaling activity is reduced in trol−/− ISCs. mys−/− ISCs lost their ability to proliferate. Moreover, the activation of integrin signaling can partially rescue the proliferation defect in trol−/− ISCs. These results suggest that trol−/− ISCs lose their ability to proliferate partially due to loss of integrin signaling activity. Our results suggest that ISC-ECM attachment is required for the activation of integrin signaling, and point out that integrin signaling plays a major role in the regulation of Drosophila ISC proliferation.

Together, we propose that ISC secrets Pcan to create an activated ECM, which is required for the niche establishment. The activated ECM is critical for both ISC-ECM attachment and the maintenance of stem cell activity and identity. Therefore, our data support a view that stem cells can generate their niches by themselves.

EXPERIMENTAL PROCEDURES

Clonal Analysis and Feeding Experiments

For clonal analysis, flies of appropriate genotypes were given heat-shock treatment at 3 days after eclosure and then kept at 25°C. For feeding experiments, 3 days ACI, flies were transferred to an empty vial with six pieces of round chromatography paper (Fisher Scientific). A total of 400 μl 5% sucrose solution or 3% DSS (MP Biomedicals) in 5% sucrose solution was added to the paper. Flies were transferred to new vials every day and were dissected 4 days after feeding with DSS.

Immunofluorescence Staining and Microscope

Antibody staining was performed as described (Belenkaya et al., 2002). After antibody staining, samples were washed in PBS with DAPI (Sigma-Aldrich) for 1 hr. Samples were observed with a Nikon
A1R si laser-scanning confocal microscope on a Nikon Ti inverted microscope. All images were taken at multiple optical sections and converted to volume view by NIS element version 4.0.

SUPPLEMENTAL INFORMATION

Supplemental Information includes four figures and Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.stemcr.2014.04.007.

ACKNOWLEDGMENTS

We thank A. Voigt, N. Perrimon, S.X. Hou, X. Zeng, H. Sun, G.H. Beag, and X. Yang for reagents; the Bloomington Stock Center and Drosophila Genetic Resource Center for Drosophila stocks; J.M. Kron for help with confocal images; and James Wells, Tatyana Belenkaya, and Lorraine Ray for comments on the manuscript. This work was supported by grants from the Strategic Priority Research Program of China (2011CB943901 and 2011 CB943902), Mizutani Foundation (120146), NIH (2R01 GM063891 and 1R01GM087517), and Wenzhou Medical University (XNK07005).

REFERENCES

Amcheslavsky, A., Jiang, J., and Ip, Y.T. (2009). Tissue damage-induced intestinal stem cell division in Drosophila. Cell Stem Cell 4, 49–61.

Bach, E.A., Ekas, L.A., Ayala-Camargo, A., Flaherty, M.S., Lee, H., Perrimon, N., and Baeg, G.H. (2007). GFP reporters detect the activation of the GPR124 in vivo. Gene Expr. Patterns 7, 323–331.

Beebe, K., Lee, W.C., and Micchelli, C.A. (2010). JAK/STAT pathway in vivo. Gene Expr. Patterns 10, 451–461.

Belenkaya, T.Y., Han, C., Standley, H.J., Lin, X., Houston, D.W., Heasman, J., and Lin, X. (2002). pygopus Encodes a nuclear protein essential for wingless/Wnt signaling. Development 129, 4089–4101.

Brown, N.H. (2000). Cell-cell adhesion via the ECM: integrin genetics in fly and worm. Matrix Biol. 19, 191–201.

Chen, S., Lewallen, M., and Xie, T. (2013). Adhesion in the stem cell niche: biological roles and regulation. Development 140, 255–265.

Chiarini-Garcia, H., Raymer, A.M., and Russell, L.D. (2003). Random distribution of spermatogonia in rats: evidence of niches in the seminiferous tubules. Reproduction 126, 669–680.

Cohen, I.R., Grässel, S., Murdoch, A.D., and Iozzo, R.V. (1993). Structural characterization of the complete human perlecan gene and its promoter. Proc. Natl. Acad. Sci. USA 90, 10404–10408.

Collins, C.A., Olsen, I., Zammit, P.S., Petrie, A., Partridge, T.A., and Morgan, J.E. (2005). Stem cell function, self-renewal, and behavioral heterogeneity of cells from the adult muscle satellite cell niche. Cell 122, 289–301.

Ellis, S.J., and Tanentzapf, G. (2010). Integrin-mediated adhesion and stem-cell-niche interactions. Cell Tissue Res. 339, 121–130.

Friedrich, M.V., Schneider, M., Timpl, R., and Baumgartner, S. (2000). Perlecan domain V of Drosophila melanogaster. Sequence, recombinant analysis and tissue expression. Eur. J. Biochem. 267, 3149–3159.

Fuchs, E., Tumbar, T., and Guasch, G. (2004). Socializing with the neighbors: stem cells and their niche. Cell 116, 769–778.

Gabay, L., Seger, R., and Shilo, B.Z. (1997). MAP kinase in situ activation atlas during Drosophila embryogenesis. Development 124, 3535–3541.

Hayashi, Y., Sexton, T.R., Dejima, K., Perry, D.W., Takemura, M., Kobayashi, S., Nakato, H., and Harrison, D.A. (2012). Glypicans regulate JAK/STAT signaling and distribution of the Unpaired morphogen. Development 139, 4162–4171.

Hynes, R.O. (2002). Integrins: bidirectional, allosteric signaling machines. Cell 110, 673–687.

Ivanov-Dikic, L., Grönroos, E., Blaukat, A., Barth, B.U., and Dikic, I. (2000). Pyk2 and FAK regulate neurite outgrowth induced by growth factors and integrins. Nat. Cell Biol. 2, 574–581.

Jiang, H., Patel, P.H., Kohlmaier, A., Grenley, M.O., McEwen, D.G., and Edgar, B.A. (2000). MAP kinase and Stat signaling mediate regeneration and homeostasis in the Drosophila midgut. Cell 137, 1343–1355.

Jiang, H., Grenley, M.O., Bravo, M.J., Blumhagen, R.Z., and Edgar, B.A. (2011). EGFR/Ras/ERK signaling mediates adult midgut epithelial homeostasis and regeneration in Drosophila. Cell Stem Cell 8, 84–95.

Jones, D.L., and Wagers, A.J. (2008). No place like home: anatomy and function of the stem cell niche. Nat. Rev. Mol. Cell Biol. 9, 11–21.

Kallunki, P., Eddy, R.L., Byers, M.G., Kestilä, M., Shows, T.B., and Tryggvason, K. (1991). Cloning of human heparan sulfate proteoglycan core protein, assignment of the gene (HSPG2) to 1p36.1——p35 and identification of a BamHI restriction fragment length polymorphism. Genomics 11, 389–396.

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 beta1-integrin. Cell Stem Cell 3, 533–542.

Kuang, S., Gillespie, M.A., and Rudnicki, M.A. (2008). Niche regulation of muscle satellite cell self-renewal and differentiation. Cell Stem Cell 2, 22–31.

Lander, A.D., Kimble, J., Clevers, H., Fuchs, E., Monttarras, D., Buckingham, M., Calof, A.L., Trumpf, A., and Oskarsson, T. (2012). What does the concept of the stem cell niche really mean today? BMC Biol. 10, 19.

Lee, T., and Luo, L. (1999). Mosaic analysis with a repressible cell marker for studies of gene function in neuronal morphogenesis. Neuron 22, 451–461.
Lin, X. (2004). Functions of heparan sulfate proteoglycans in cell signaling during development. Development 131, 6009–6021.

Liu, W., Singh, S.R., and Hou, S.X. (2010). JAK-STAT is restrained by Notch to control cell proliferation of the Drosophila intestinal stem cells. J. Cell. Biochem. 109, 992–999.

Lucchetta, E.M., and Ohlstein, B. (2012). The Drosophila midgut: a model for stem cell driven tissue regeneration. Wiley Interdiscip. Rev. Dev. Biol. 1, 781–788.

Maeda, K., Takemura, M., Umemori, M., and Adachi-Yamada, T. (2008). E-cadherin prolongs the moment for interaction between intestinal stem cell and its progenitor cell to ensure Notch signaling in adult Drosophila midgut. Genes Cells 13, 1219–1227.

Marthiens, V., Kazanis, I., Moss, L., Long, K., and Ffrench-Constant, C. (2010). Adhesion molecules in the stem cell niche—more than just staying in shape? J. Cell Sci. 123, 1613–1622.

Michelli, C.A., and Perrimon, N. (2006). Evidence that stem cells reside in the adult Drosophila midgut epithelium. Nature 439, 475–479.

Morrison, S.J., and Spradling, A.C. (2008). Stem cells and niches: mechanisms that promote stem cell maintenance throughout life. Cell 132, 598–611.

Ohlstein, B., and Spradling, A. (2006). The adult Drosophila posterior midgut is maintained by pluripotent stem cells. Nature 439, 470–474.

Pastor-Pareja, J.C., and Xu, T. (2011). Shaping cells and organs in Drosophila by opposing roles of fat body-secreted Collagen IV and perlecan. Dev. Cell 21, 245–256.

Ren, F., Wang, B., Yue, T., Yun, E.Y., Ip, Y.T., and Jiang, J. (2010). Hippo signaling regulates Drosophila intestine stem cell proliferation through multiple pathways. Proc. Natl. Acad. Sci. USA 107, 21064–21069.

Schneider, M., Khalil, A.A., Poulton, J., Castillejo-Lopez, C., Egger-Adam, D., Wodarz, A., Deng, W.M., and Baumgartner, S. (2006). Perlecan and Dystroglycan act at the basal side of the Drosophila follicular epithelium to maintain epithelial organization. Development 133, 3805–3815.

Shen, Q., Wang, Y., Kokovay, E., Lin, G., Chuang, S.M., Goderie, S.K., Roysam, B., and Temple, S. (2008). Adult SVZ stem cells lie in a vascular niche: a quantitative analysis of niche cell-cell interactions. Cell Stem Cell 3, 289–300.

Voigt, A., Pflanz, R., Schafer, U., and Jackle, H. (2002). Perlecan participates in proliferation activation of quiescent Drosophila neuroblasts. Dev. Dyn. 224, 403–412.

Watt, F.M. (2002). Role of integrins in regulating epidermal adhesion, growth and differentiation. EMBO J. 21, 3919–3926.

Xie, T., and Spradling, A.C. (2000). A niche maintaining germ line stem cells in the Drosophila ovary. Science 290, 328–330.

Xu, N., Wang, S.Q., Tan, D., Gao, Y., Lin, G., and Xi, R. (2011). EGFR, Wingless and JAK/STAT signaling cooperatively maintain Drosophila intestinal stem cells. Dev. Biol. 354, 31–43.

Zeng, X., Lin, X., and Hou, S.X. (2013). The Osa-containing SWI/SNF chromatin-remodeling complex regulates stem cell commitment in the adult Drosophila intestine. Development 140, 3532–3540.

Zhang, Y., You, J., Ren, W., and Lin, X. (2013). Drosophila glypicans Dally and Dally-like are essential regulators for JAK/STAT signaling and Unpaired distribution in eye development. Dev. Biol. 375, 23–32.