· Advances in Medical Sciences · Vol. 54(2) · 2009 · pp 143-149 · DOI: 10.2478/v10039-009-0032-5

Stem cell niche in the *Drosophila* ovary and testis; a valuable model of the intercellular signalling relationships

Pałasz A*, Kamiński M

Department of Histology, Medical University of Silesia, Katowice, Poland

* CORRESPONDING AUTHOR:
Department of Histology,
Medical University of Silesia,
ul. Medyków 18,
40-752 Katowice, Poland.
Phone; (032)2088377, (032)2526574,
e-mail: apalasz@slam.katowice.pl (Artur Pałasz)

Received 06.07.2009
Accepted 12.08.2009
Advances in Medical Sciences
Vol. 54(2) · 2009 · pp 143-149
DOI: 10.2478/v10039-009-0032-5

ABSTRACT

One of the key factors determining the function of all types of stem cells is their location in a specific microenvironment called a niche which is understood as a system of adjacent cells directly influencing their ability to carry out self-renewal divisions. The cells which compose the niche influence cytophysiological processes of stem cells both directly via the intercellular junction system and via the synthesis and release of many protein regulatory substances which are ligands of specific receptors in a particular stem cell. These proteins are often the products of distinct genes whose expression tends to be specific for niche-composing cells. The niches formed of a few cells only observed in Drosophila gonads may become a valuable functional model in the studies of mammal stem cells since their analysis proves that the preservation of the stem cells' unique features does not require a large number of cells to be present in its vicinity.

Key words: stem cell niche, oogenesis, spermatogenesis, progenitor cells

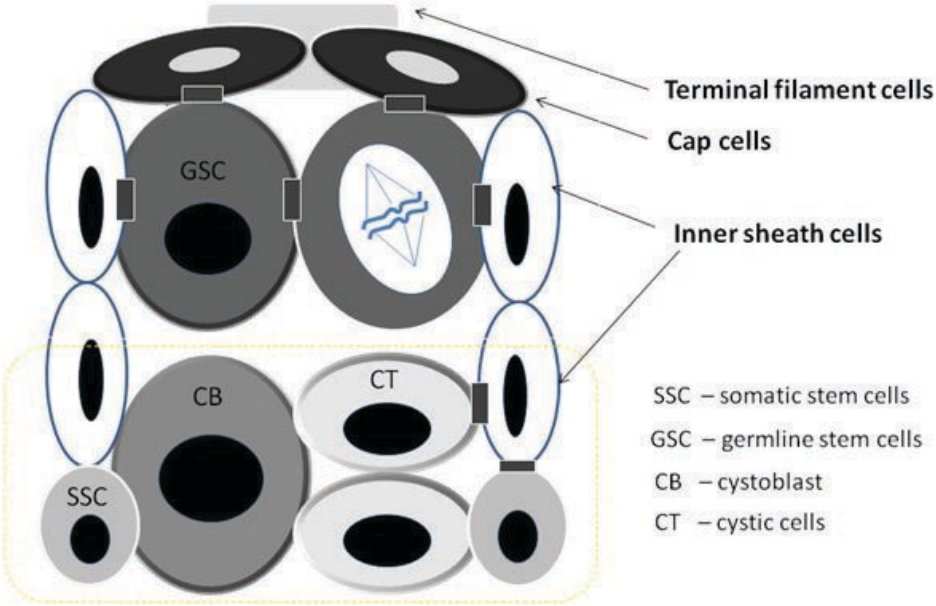
INTRODUCTION

The unique nature of cells derives from their ability to carry out self-renewal divisions which result in the preservation of an undifferentiated cell population. This group of cells plays a crucial role in the stabilization of the organ structure, determines the reparation and regeneration of damaged tissues, and enables the preservation of intercellular homeostasis [1,2]. Responsible for a continuous haploid germ cell production process, germline stem cells found in gonads have a special function to perform. Widespread studies on stem cell properties in recent years have primarily focused on explaining regulatory mechanisms which those cells undergo, and on showing and evaluating the expression of the genes and proteins which are specific for them, formed due to the translation process. The ability of stem cells to renew themselves with the simultaneous active inhibition of their differentiation can be sustained owing to the specific cellular and humoral microenvironment where they are found, referred to as a stem cell niche [3].

The niches concentrate in restricted and at the same time highly specialized organ fragments. Consequently, in the stomach they are located in the isthmus of the main glands [4]; in the small intestine between the fourth and eighth layer of the intestinal crypt cells [5]; in the large intestine also in the crypts, but in the first or the second stratum [6]; in the liver near Hering's canaliculi [7-9]; in the pancreas usually around or in the epithelium covering intercalated ducts [10,11]; in the skin inside the bulge of the hair follicles, close to the sebaceous glands [12]; and in male gonads in the direct vicinity of the supportive Sertoli cells [13,14]. In the CNS, the neural stem cell niche seems to be located beneath the ependymal cell layer that lines the brain ventricles, within so called subventicular zone (SVZ) as well as in the dentate gyrus. [15,16]. The stem cell niches may be also dispersed in the excretory ducts of the mammary gland [17,18].

Niche cells influence stem cells in a paracrine or cytocrine manner, and directly through intercellular gap junctions, causing a series of biochemical effects which are manifested by the activation or inhibition of certain genes, and, as a consequence, by their proteome modification. In these interactions, specific stem cell receptors perform a primary function. The internal environment of the niche is also modified via endocrine-like signals generated outside the niche or neurotransmitters released from nerve endings [19].

Figure 1. **Schematic representation of the ovarian stem cell niche in *Drosophila*.**



The term of a stem cell niche was first used with reference to the cells surrounding pluripotent haematopoietic cells [20-22]. Studies on a bone marrow niche as well as the niches in other organs have encountered significant problems resulting from the complexity of the niche forming cell composition, high proliferation dynamics and, above all, difficulties in the precise separation of the cell system composing a single niche. Under these conditions, it is not easy to identify repetitive groups of niche cells, constant in terms of their composition, in which the same model of interactions with stem cells occurs. Therefore, it seems valuable to search for simpler niche models of stem cells, based on systems formed from a small, usually constant number of cells. These conditions are perfectly met by stem cell niches located in gonads of invertebrate organisms, including insects represented by *Drosophila melanogaster*, a model organism in molecular biology [23].

REVIEW

The ovarian niche of *Drosophila melanogaster* is found in the apical blind end of an elongated tubular structure. It is responsible for the production of mature gametes which are released from the basal ovarian end. The ovarian unit called an ovariole contains 2-3 germinal stem cells located in a specialized structure called the germarium. The niche is formed by terminal filament cells on the germarium apex and cap cells which are in contact with underlying stem cells. Dividing asymmetrically, germline stem cells produce two sisters, of which the first remains in contact with the cap cell, while the other, called a cytoblast, loses this contact and disappears via the differentiation (*Fig. 1*). Recent studies suggest that the Sevenless (Sev) receptor, present in somatic cells of the posterior ovary region, is responsible for the proper niche location in the apical pole of an ovariole, just like its Boss (Bride of Sevenless) ligand released by germline cells [24]. Following the stimulation of that receptor, the tyrosine kinase pathway activation prevents ectopic stem cell niche formation. In the case of a testicular niche, however, there is a set of somatic cells called hub cells located in the apical region of the organ. Morphological and genetic analyses reveal 5-9 germinal stem cells in the testicle of a mature specimen, which are still in contact with central cells and form a concentric ring around them [25].

Each germinal stem cell is flanked by a pair of progenitor cells: somatic stem cells which also remain in contact with central cells. The germline stem cell and two accompanying somatic cells divide asymmetrically in a radial orientation in relation to the centre, creating differentiated sister cells. Those from the cells which have been formed from germinal cells, called gonialblasts, divide further and produce interconnected spermatogonia, while the two differentiated somatic cells do not divide but grow inside spermatogonia cysts [26]. New germline stem cells and gonial-blasts are connected by cytoplasmatic bridges which are preserved during the next division. This is reminiscent of the situation observed during a stem cell division both in the ovaries of the drosophila and the testicles of mammals.

An analysis of the ovarian and testicular niche structure and function shows both plenty of similarities but also significant differences occurring between them. A key role in the niches is played by specific accompanying cells which not only surround stem cells, creating a specific humoral microenvironment, but also directly bind with them through a system of intercellular junctions of the *zonula adhaerens* and *nexus* type [27]. The role of these cells is the paracrine activation of signalling

pathways which ensures maintaining the stem cell phenotype and protects them from the differentiation. However, the signal transduction mechanism is very distinct in both niche types and depends on the expression of other gene groups.

In the region of the ovarian niche, cap cells play a key regulatory role. It is suggested that only these cells, with the presumed participation of cells found in the basal area of the terminal filament, are responsible for germinal stem cell survival. Cap cells synthesize and release two regulatory factors: dpp (decapentaplegic) and a BMP (bone morphogenic protein) analogue known as gbb (glass bottom boat). They stimulate the SMAD signalling pathway of the germinal stem cells, preventing their differentiation and allowing for the preservation of the self-renewal cell status [28-31]. The *dpp (decapentaplegic)* gene encodes a signalling molecule similar to the transforming growth factor beta (TGF-β). The loss of the function by *dpp* leads to germline stem cell disintegration, while the *dpp* overexpression leads to their neoplastic transformation. A clonal analysis of the receptors and messengers participating in the dpp signalling pathway, such as *punt* (an encoding dpp type II receptor) and *Mad/Med (Mother against dpp/Medea)* encoding the dimeric transcription activator, shows that the *dpp* signalling is directly received by the germline stem cells. A fundamental yet still poorly explained role in the signalling processes of the ovarian niche is played by *Yb*, *hh* (hedgehog) and *en* (engrailed) genes which show expression only in cap and terminal filament cells [32-36]. The loss of the *Yb* function causes stem cells to differentiate directly, without divisions into the germinal cyst line. They might as well undergo a limited number of pathological mitoses, losing their self-renewal ability. The inhibition of the *hh* expression during oogenesis affects germinal stem cells to a small degree, but the *en* participation in that process has not been explained yet.

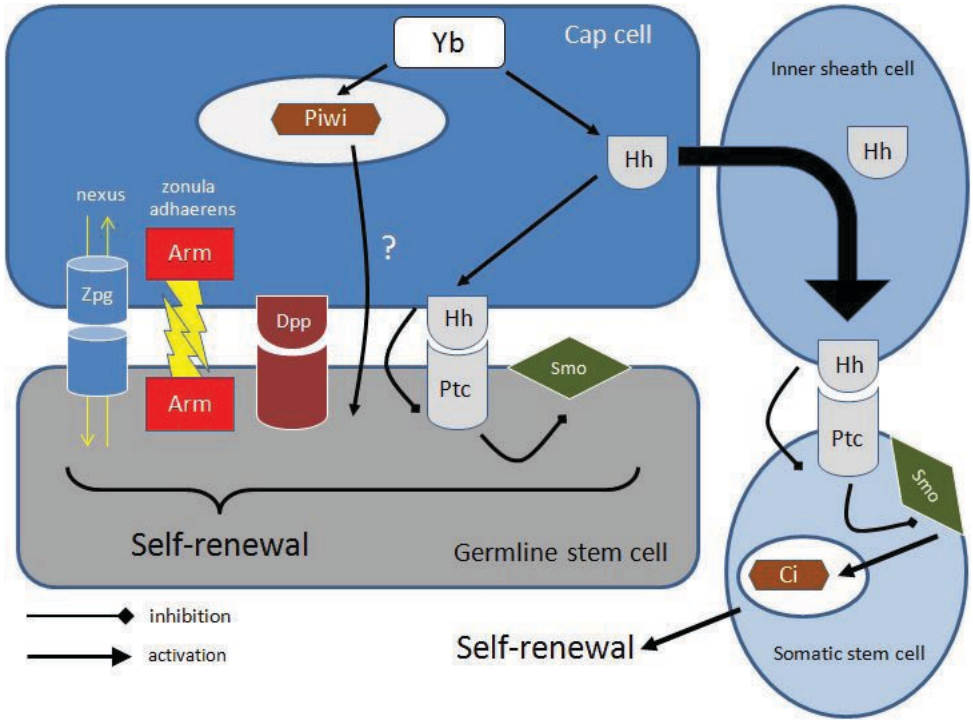
An important role in maintaining the stem cell line is also played by the *piwi* gene which shows expression both in germinal and somatic cells, including cap and filament cells. It belongs to the *Argonaute* gene family which encodes alkaline proteins analogous to EIF2C (a factor initiating translation in eukaryotes) and plays a key role in the stem cell division, gametogenesis and RNA muffling in animal and plant organisms [37,38]. Undoubtedly, Yb and Piwi are elements of signalling pathways other than dpp. This is also supported by the following findings. Firstly, the Piwi and Hh expression in cap and filament cells depends on Yb, which does not refer to the dpp expression since it is not regulated. Secondly, although the *piwi* and *Yb* elimination leads to a stem cell disintegration similar to that observed in the absence of *dpp*, the *Yg* or *piwi* overexpression in somatic cells doubles the number of germline stem cells. The signal dependent on *Yb/piwi* from the niche seems to modulate the rate of germline stem cell divisions in a dose dependent manner, while *dpp* functions on the basis of a different mechanism, not yet known. *Yb* and *Piwi* gene products do not show signalling molecule features and are probably not secreted by the cell, but the signalling molecule produced on the Yb/Piwi pathway is the Hh protein. This protein is a ligand located in the cellular membrane in germinal stem cells of the ptc receptor, a product of a specific *ptc (patched)* gene [39].

The connection of the Hh molecule with that receptor causes unblocking of the Smo (Smoothened) membrane protein suppression, which allows it to enter the intercellular signalling pathway and activate the Ci (*Cubitus interruptus*) factor homologous to Gli1. This leads to an initiation of the special gene transcription in the target cell [40]. The Hh protein shows a specific expression in the niche cells, and the relative signalling pathway plays a leading role in controlling the somatic stem cells division. However, its participation in mechanisms regulating germinal stem cell functions is slight. The effect of the Hh expression decrease is a poorly pronounced dysfunction in these cells, while overexpression results in a moderate increase in their number only. The expression of *hh* in the niche cells requires Yb; however, it is independent from Piwi, indicating that Hh and Piwi represent bifurcated arms of the Yb regulatory pathway. Phenotypically, ptc-deprived somatic cells resemble the cells with *hh* overexpression; yet those which lost Smo are similar to mutants with the *hh* knockout gene. Still, in the *Drosophila* ovary somatic stem cells do not come into direct contact with the cap cells, but they are separated by a group of postmitotic epithelial cells, called coating cells. Therefore, how does signalling via Hh take place in this situation?

It seems that Hh molecules produced in the cap cells can move with an elongated, peripheral niche "arm", migrating inside coating cells to reach receptors located in the somatic stem cell membrane. All of these observations tend to show that the signalling pathway dependent on Piwi controls germline stem cell divisions, while the Hh-dependent pathway regulates the mitosis process in the somatic cells. There are certain alternative arms of the Piwi signalling pathway which might generate a series of other signals important for maintaining the germline stem cells. These signals include cytokines, small-molecule substances penetrating via nexus type junctions and extracellular matrix components or proteins forming intercellular junctions. Beside *piwi,* DE-cadherin (also called *shotgun*) and β-catenin (*arm; armadillo)* show expression in all cells of the germinal and somatic lines. The significant DE-cadherin and arm role in maintaining the stem cell line pinpoints the important role of the intercellular adhesion in determining the stem cell destiny [41,42].

Signalling from the niche can also occur via the nexus junction. The *zpg* (*zero population growth)* gene encodes the protein of the *nexus* junctions, innexin 4, which is specific for germinal line stem cells [43,44]. This protein is anchored in cellular membranes, in the places of contact with the cap and somatic cells. The *zpg-null* mutants are marked by a constant number of abnormal germinal cells which may gradually replace stem cells or their sister cells. Therefore, zpg is necessary for early terminal cell survival and differentiation. The RanBPM protein also plays an important role in the niche functioning as it binds the molecules of the Ran protein which is responsible

Figure 2. **A model of signalling pathways in the ovarian stem cell niche of *Drosophila*. (based on Lin H. 2002 et al., modified). The main intercellular interactions: Arm; Armadillo, Ci; Cubitus interruptus, Dpp; Decapentaplegic, Hh; Hedgehog, Ptc; Patched, Smo; Smoothened, Yb; female sterile (1) Yb, Zpg; zero population growth – innexin 4 (see text for details).**



for the two-directional transport between the nucleus and cytoplasm. Thus, the ovarian niche of the stem cells has two features: first, it produces numerous regulatory molecules responsible for germinal cell line maintenance, and second, it ensures intercellular adhesion. It is worth emphasizing that the niche is in partial balance of the opposing interactions: in the proximal part (cap cells), processes responsible for self-renewal abilities of the stem cells dominate, and in the distal part (filament cells) stem cells division suppression and differentiation activation occur (*Fig. 2*).

Furthermore, key signalling molecules of the *Drosophila* central testicular niche release an Unp (Unpaired) ligand which activates the specific Janus kinase, also known as Hop; Hopscotch [46]. The Jak-Stat transduction pathway stimulation activates the transcription in the adjacent stem cells and maintains their self-renewal process [47,48]. The loss of Unp, Jak or Stat results in disorders in stem cell maintenance, while excessive Jak-Stat pathway activation caused by *Upd* ectopic expression leads to an unlimited stem cell amplification. Ovarian and testicular niches differ in the *dpp* pathway role during oogenesis and spermatogenesis, respectively. Firstly, the *dpp* signalling does not occur in the maintenance or division of nuclear germline stem cells, but it limits the proliferation of differentiated sister cells. Secondly, testicular germinal cells do not require *dpp* signal transmitters and receptors, as opposed to somatic cells. The loss of the *punt* and *shn (shnurri)* function in somatic cells of the cysts leads to an excessive proliferation of gonial-blasts and their sister cells, not causing that effect in germline stem cells. *Shn* encodes the transcription factor with a zinc finger motif (a BMP1/2 homologue), which is down-regulated by the *dpp* signal, affecting dependent genes expression. The *dpp* influence on germinal cells is not direct, and it is accomplished via somatic cells. Another signalling pathway limiting the germline stem cell proliferation in the testicle works via *raf (pole hole)*. This self-renewal restriction and control of the germinal stem cell number occurs with the participation of the *raf* somatic cells. Since *raf* is a key component of the Ras/ERK pathway (signal regulated intercellular kinase), it can be assumed that the Ras/Raf/ERK system plays a primary role in limiting stem cell proliferation [23].

The receptor for the *raf* pathway has not been identified yet, but it is known that the epidermal growth factor receptor (Egfr) limits excessive stem cell proliferation in the testis. The loss of Egfr in somatic cells disturbs the balance between stem cell self-renewal and differentiation, leading to an increase in the number of stem cells. Raf and Egfr function in the same cells causing similar effects, thus, the stimulation of Egfr activates the Ras/Raf/ERK pathway. An important role in shaping the testicular stem cell niche seems to be played also by the Lasp-1 protein which is one of adhesive molecules responsible for cell migration [49]. In recent years, some stem cell niches of vertebrates have successfully been described. The closest to the gonad niche of the *Drosophila*, at least in structural terms, seems to be the stem cell niche, located in the hair follicle bulge of humans and rodents. It has been

found that the niche functioning relies on Wnt and Shh (Sonic Hedgehog) signalling pathways [50]. The morphology and cytophysiology of a bone marrow cell niche have not been satisfactorily defined yet, however, it is known that in mice, the signalling pathway of Steel/c-Kit as well as Wnt, Shh and Notch pathways play a key role in hematopoiesis [51-54]. Furthermore, in the niche found in mice intestinal crypts, the expression of B1 ephrine and its membrane receptors, EphB2 and EphB3, has been revealed [55].

CONCLUSIONS

Information obtained so far, concerning the structure and function of the gonad stem cell niche of the *Drosophila*, allows for generalizations which, in their basic formulation, might be referred to as the niches found in the organs of vertebrates. Firstly, mammals' niches do not have to be a complex and intricate set of cells to play their function. Probably, a few stromal cells only or even just one of them may be sufficient to control the division of bone marrow or hair follicle stem cells. Secondly, mammals' niches are distinguished by a functional duality, for instance, bone marrow stromal cells can control the division of both hematopoietic and mesenchymal progenitor cells. Furthermore, connective tissue cell clusters of the olfactory section in the nose can regulate both epithelial and neural stem cell divisions. Thirdly, this can also be found in mammals with the same signalling pathway participating in various stem cell systems, but its function in each of the systems is different. In mammals, the Dpp homologue, BMP8B, is necessary for germline stem cell initiation and maintenance, similarly to Dpp in *Drosophila* oogenesis. However, differently from insects, BMP8B shows expression in germinal cells, and not in niche cells. Although the Jak-Stat signalling pathway generally occurs in embryonic stem cell maintenance in mammals, it can probably perform alternative functions in tissue stem cell systems. On the other hand, the Steel/c-Kit pathway necessary to maintain self-renewal of hematopoietic stem cells can stimulate the differentiation of seminiferous epithelium stem cells. Besides, it can also be assumed that mammal stem cell niches utilize the mechanisms which are highly tissue-specific. It has been observed that a signal coming from the niche can selectively influence the transcription of specific genes in particular stem cell types.

There is evidence that the stem cell niche in mammals not only regulates its self-renewal potential, but it also restricts the differentiation range to the tissue where it takes place. For example, a single isolated embryonic stem cell often shows higher differentiation flexibility compared to the mass of those cells *in situ*; under *in vitro* conditions, liver stem cells can undergo transdifferentiation to pancreatic endocrine cells. In addition, it is not known how the niche restricts the stem cell differentiation potential. Probably, mammal stem cells begin apoptosis as a standard mechanism to eliminate the excess of stem cells and their differentiated sister cells. Thus, many questions remain unanswered; however, large scale studies on the gonadal niches of the *Drosophila* have provided and continue to provide plenty of surprising as well as exciting information concerning their structure and, what is more, the molecular mechanisms of their activity, which sheds new light on the theory of stem cell niches which continues to be formulated.

REFERENCES

1. Hall PA, Watt FM. Stem cells: the generation and maintenance of cellular diversity. Development. 1989 Aug;106(4):619-33.
2. Jones DL, Wagers AJ. No place like home: anatomy and function of the stem cell niche. Nat Rev Mol Cell Biol. 2008 Jan;9(1):11-21.
3. Watt FM, Hogan BL. Out of Eden: stem cells and their niches. Science. 2000 Feb 25;287(5457):1427-30.
4. Yen TH, Wright NA. The gastrointestinal tract stem cell niche. Stem Cell Rev. 2006;2(3):203-12.
5. Clarke AR, Meniel V. The intestinal stem cell niche studied through conditional transgenesis. Ernst Schering Found Symp Proc. 2006;(5):99-108.
6. Radford IR, Lobachevsky PN. An enteroendocrine cell-based model for a quiescent intestinal stem cell niche. Cell Prolif. 2006 Oct;39(5):403-14.
7. Kuwahara R, Kofman AV, Landis CS, Swenson ES, Barendswaard E, Theise ND. The hepatic stem cell niche: identification by label-retaining cell assay. Hepatology. 2008 Jun;47(6):1994-2002.
8. Theise ND. Gastrointestinal stem cells. III. Emergent themes of liver stem cell biology: niche, quiescence, self-renewal, and plasticity. Am J Physiol Gastrointest Liver Physiol. 2006 Feb;290(2):G189-93.
9. Theise ND, Saxena R, Portmann BC, Thung SN, Yee H, Chiriboga L, Kumar A, Crawford JM. The canals of Hering and hepatic stem cells in humans. Hepatology. 1999 Dec;30(6):1425-33.
10. Burke ZD, Thowfeequ S, Peran M, Tosh D. Stem cells in the adult pancreas and liver. Biochem J. 2007 Jun 1;404(2):169-78.
11. Yalniz M, Pour PM. Are there any stem cells in the pancreas? Pancreas. 2005 Aug;31(2):108-18.
12. Lavker RM, Sun TT, Oshima H, Barrandon Y, Akiyama M, Ferraris C, Chevalier G, Favier B, Jahoda CA, Dhouailly D, Panteleyev AA, Christiano AM. Hair follicle stem cells. J Investig Dermatol Symp Proc. 2003 Jun;8(1):28-38.
13. Goossens E, Tournaye H. Testicular stem cells. Semin Reprod Med. 2006 Nov;24(5):370-8.
14. Shinohara T, Orwig KE, Avarbock MR, Brinster RL. Restoration of spermatogenesis in infertile mice by Sertoli cell transplantation. Biol Reprod. 2003 Mar;68(3):1064-71.
15. Conover JC, Notti RQ. The neural stem cell niche. Cell Tissue Res. 2008 Jan;331(1):211-24.
16. Quiñones-Hinojosa A, Sanai N, Soriano-Navarro

M, Gonzalez-Perez O, Mirzadeh Z, Gil-Perotin S, Romero-Rodriguez R, Berger MS, Garcia-Verdugo JM, Alvarez-Buylla A. Cellular composition and cytoarchitecture of the adult human subventricular zone: a niche of neural stem cells. J Comp Neurol. 2006 Jan 20;494(3):415-34.

17. Chepko G, Dickson RB. Ultrastructure of the putative stem cell niche in rat mammary epithelium. Tissue Cell. 2003 Apr;35(2):83-93.

18. Dontu G, Al-Hajj M, Abdallah WM, Clarke MF, Wicha MS. Stem cells in normal breast development and breast cancer. Cell Prolif. 2003 Oct;36 Suppl 1:59-72.

19. Scadden DT. The stem-cell niche as an entity of action. Nature. 2006 Jun 29;441(7097):1075-9.

20. Frisch BJ, Porter RL, Calvi LM. Hematopoietic niche and bone meet. Curr Opin Support Palliat Care. 2008 Sep;2(3):211-7.

21. Crozatier M, Krzemien J, Vincent A.The hematopoietic niche: a Drosophila model, at last. Cell Cycle. 2007 Jun 15;6(12):1443-4.

22. Yaniv I, Stein J, Farkas DL, Askenasy N. The tale of early hematopoietic cell seeding in the bone marrow niche. Stem Cells Dev. 2006 Feb;15(1):4-16.

23. Lin H. The stem-cell niche theory: lessons from flies. Nat Rev Genet. 2002 Dec;3(12):931-40.

24. Kitadate Y, Shigenobu S, Arita K, Kobayashi S. Boss/Sev signaling from germline to soma restricts germline-stem-cell-niche formation in the anterior region of Drosophila male gonads. Dev Cell. 2007 Jul;13(1):151-9.

25. Voog J, D'Alterio C, Jones DL. Multipotent somatic stem cells contribute to the stem cell niche in the Drosophila testis. Nature. 2008 Aug 28;454(7208):1132-6.

26. Le Bras S, Van Doren M. Development of the male germline stem cell niche in Drosophila. Dev Biol. 2006 Jun 1;294(1):92-103.

27. Song X, Zhu CH, Doan C, Xie T. Germline stem cells anchored by adherens junctions in the Drosophila ovary niches. Science. 2002 Jun 7;296(5574):1855-7.

28. Xie T, Spradling AC. Decapentaplegic is essential for the maintenance and division of germline stem cells in the Drosophila ovary. Cell. 1998 Jul 24;94(2):251-60.

29. Chen D, McKearin D. Dpp signaling silences bam transcription directly to establish asymmetric divisions of germline stem cells. Curr Biol. 2003 Oct 14;13(20):1786-91.

30. Zhu CH, Xie T. Clonal expansion of ovarian germline stem cells during niche formation in Drosophila. Development. 2003 Jun;130(12):2579-88.

31. Song X, Wong MD, Kawase E, Xi R, Ding BC, McCarthy JJ, Xie T. Bmp signals from niche cells directly repress transcription of a differentiation-promoting gene, bag of marbles, in germline stem cells in the Drosophila ovary. Development. 2004 Mar;131(6):1353-64.

32. Szakmary A, Cox DN, Wang Z, Lin H. Regulatory relationship among piwi, pumilio, and bag-of-marbles in Drosophila germline stem cell self-renewal and differentiation. Curr Biol. 2005 Jan 26;15(2):171-8.

33. Bolívar J, Pearson J, López-Onieva L, González-Reyes A. Genetic dissection of a stem cell niche: the case of the Drosophila ovary. Dev Dyn. 2006 Nov;235(11):2969-79.

34. Chen D, McKearin D. Gene circuitry controlling a stem cell niche. Curr Biol. 2005 Jan 26;15(2):179-84.

35. Zhang Y, Kalderon D. Hedgehog acts as a somatic stem cell factor in the Drosophila ovary. Nature. 2001 Mar 29;410(6828):599-604.

36. Takashima S, Mkrtchyan M, Younossi-Hartenstein A, Merriam JR, Hartenstein V. The behaviour of Drosophila adult hindgut stem cells is controlled by Wnt and Hh signalling. Nature. 2008 Jul 31;454(7204):651-5.

37. Höck J, Meister G. The Argonaute protein family. Genome Biol. 2008;9(2):210.

38. Deshpande G, Calhoun G, Schedl P. Drosophila argonaute-2 is required early in embryogenesis for the assembly of centric/centromeric heterochromatin, nuclear division, nuclear migration, and germ-cell formation. Genes Dev. 2005 Jul 15;19(14):1680-5.

39. Zugasti O, Rajan J, Kuwabara PE. The function and expansion of the Patched- and Hedgehog-related homologs in C. elegans. Genome Res. 2005 Oct;15(10):1402-10.

40. Shyamala BV, Bhat KM. A positive role for patched-smoothened signaling in promoting cell proliferation during normal head development in Drosophila. Development. 2002 Apr;129(8):1839-47.

41. Tanentzapf G, Devenport D, Godt D, Brown NH. Integrin-dependent anchoring of a stem-cell niche. Nat Cell Biol. 2007 Dec;9(12):1413-8.

42. Song X, Xie T. DE-cadherin-mediated cell adhesion is essential for maintaining somatic stem cells in the Drosophila ovary. Proc Natl Acad Sci U S A. 2002 Nov 12;99(23):14813-8.

43. Stebbings LA, Todman MG, Phillips R, Greer CE, Tam J, Phelan P, Jacobs K, Bacon JP, Davies JA. Gap junctions in Drosophila: developmental expression of the entire innexin gene family. Mech Dev. 2002 May;113(2):197-205.

44. Gilboa L, Forbes A, Tazuke SI, Fuller MT, Lehmann R. Germ line stem cell differentiation in Drosophila requires gap junctions and proceeds via an intermediate state. Development. 2003 Dec;130(26):6625-34.

45. Dansereau DA, Lasko P. RanBPM regulates cell shape, arrangement, and capacity of the female germline stem cell niche in Drosophila melanogaster. J Cell Biol. 2008 Sep 8;182(5):963-77.

46. Harrison DA, McCoon PE, Binari R, Gilman M, Perrimon N. Drosophila unpaired encodes a secreted protein that activates the JAK signaling pathway. Genes Dev. 1998 Oct 15;12(20):3252-63.

47. Kiger AA, Jones DL, Schulz C, Rogers MB, Fuller MT. Stem cell self-renewal specified by JAK-STAT activation in response to a support cell cue. Science. 2001 Dec 21;294(5551):2542-5.

48. Tulina N, Matunis E. Control of stem cell self-renewal in Drosophila spermatogenesis by JAK-STAT

signaling. Science. 2001 Dec 21;294(5551):2546-9.

49. Lee S, Zhou L, Kim J, Kalbfleisch S, Schöck F. Lasp anchors the Drosophila male stem cell niche and mediates spermatid individualization. Mech Dev. 2008 Sep-Oct;125(9-10):768-76.

50. Reddy S, Andl T, Bagasra A, Lu MM, Epstein DJ, Morrisey EE, Millar SE. Characterization of Wnt gene expression in developing and postnatal hair follicles and identification of Wnt5a as a target of Sonic hedgehog in hair follicle morphogenesis. Mech Dev. 2001 Sep;107(1-2):69-82.

51. Kent DG, Dykstra BJ, Cheyne J, Ma E, Eaves CJ. Steel factor coordinately regulates the molecular signature and biologic function of hematopoietic stem cells. Blood. 2008 Aug 1;112(3):560-7.

52. Nakamura Y, Tajima F, Ishiga K, Yamazaki H, Oshimura M, Shiota G, Murawaki Y. Soluble c-kit receptor mobilizes hematopoietic stem cells to peripheral blood in mice. Exp Hematol. 2004 Apr;32(4):390-6.

53. Feng Z, Srivastava AS, Mishra R, Carrier E. A regulatory role of Wnt signaling pathway in the hematopoietic differentiation of murine embryonic stem cells. Biochem Biophys Res Commun. 2004 Nov 26;324(4):1333-9.

54. Duncan AW, Rattis FM, DiMascio LN, Congdon KL, Pazianos G, Zhao C, Yoon K, Cook JM, Willert K, Gaiano N, Reya T. Integration of Notch and Wnt signaling in hematopoietic stem cell maintenance. Nat Immunol. 2005 Mar;6(3):314-22.

55. Holmberg J, Genander M, Halford MM, Annerén C, Sondell M, Chumley MJ, Silvany RE, Henkemeyer M, Frisén J. EphB receptors coordinate migration and proliferation in the intestinal stem cell niche. Cell. 2006 Jun 16;125(6):1151-63.