Chapter 6
Signaling Pathways Regulating Stem Cells

Xingting Guo, Jun Chen, Zhaohui Li and Rongwen Xi

Abstract Stem cells have a unique ability to self-renewal and a potential to differentiate into one or more types of specialized cells. These properties in vivo are commonly maintained and regulated by signaling pathways involved in cell-to-cell communications. Signaling communications occur between stem cells and the niche supporting cells, as well as between stem cells and their differentiated daughter cells. Different types of stem cells within the same tissue are also coordinately regulated by signaling pathways to maintain tissue architecture and function. Signaling pathways are also critical in mediating stem cell activation in response to tissue damage for accelerated regeneration. This chapter will review signaling mechanisms in controlling various behaviors of several well-characterized tissue stem cells, including self-renewal, differentiation, and regenerative activation of stem cells.

Keywords Stem cell · Signaling pathway · Mechanisms

6.1 Introduction

Adult stem cells or tissue stem cells have been a research of focus for many years, due to their prominent roles in a variety of biological processes, including tissue homeostasis, regeneration, organogenesis, and tumorigenesis. Stem cells are a population of undifferentiated cells that can self-renew via mitosis and differentiate into specialized progenies. There are many types of adult stem cells, such as hematopoietic stem cells (HSCs), neuronal stem cells (NSCs), intestinal stem cells (ISCs), and germline stem cells (GSCs) (Li and Xie 2005; Gancz and Gilboa 2013). Tightly controlled proliferation and differentiation of these stem cells

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throughout adulthood is critical for normal tissue homeostasis and damage repair. Once such regulation is disrupted, uncontrolled stem cells may lead to tumor initiation or stem cell depletion and consequently degenerative diseases (Voog and Jones 2010).

Adult stem cells commonly reside in a specialized tissue microenvironment or niche, and secreted signals from the niche have important roles in regulating stem cell maintenance, proliferation, and differentiation (Schofield 1977; Morrison and Spradling 2008). These secreted niche signals usually directly activate surface receptors on the cell membrane of stem cells, followed by the activation of signal cascades in the cytoplasm and eventually the activation of transcription factors and gene expression programs, which controls the proliferation, survival, or differentiation of stem cells. Signaling pathways are also commonly involved in mediating the response of stem cells to external stimuli (nutrient, tissue damage, and bacterial infection, etc.) to coordinate stem cell activity with demands. Recent advances in cell biology and biochemistry have largely illustrated details of these signaling pathways, and genetic analysis on model organisms such as *Drosophila melanogaster* and *Mus musculus* has provided insights into their functions in stem cell regulation. In the following parts, we will provide an overview of several commonly utilized signaling pathways and their roles in the regulation of adult stem cells.

6.2 Wnt Pathway

The Wnt signaling is highly conserved from invertebrates to vertebrates (Klaus and Birchmeier 2008). Since the discovery of the *Drosophila* segment polarity gene Wingless and its homologue Int-1 in the murine (Nusse and Varmus 1982; Rijsewijk et al. 1987), more mutations identified in *Drosophila* with similar phenotypes with $wg^{-/-}$ have led to the identification of downstream signaling components, which constitute the canonical Wnt signaling cascade (Nusse et al. 1991). Activation of the pathway requires the binding of secreted Wnt ligands to Frizzled receptors and LRP5–LRP6 co-receptors in the plasma membrane (Finch et al. 1997). The central player during Wnt signal transduction is a cytosolic protein called β-catenin. Under quiescent state, newly synthesized β-catenin binds to a destructive complex consisting of two scaffold proteins—the tumor suppressor adenomatous polyposis coli (APC) and Axin—and two kinases in this complex, CKI and GSK3, and sequentially phosphorylates a set of conserved Ser and Thr residues in the N-terminus of β-catenin afterward (Peters et al. 1999; Amit et al. 2002). The phosphorylated signal can be recognized by a b-TrCP-containing E3 ligase and lead to proteasomal degradation of β-catenin (Liu et al. 2002; Aberle et al. 1997). Meanwhile, with little β-catenin in the nucleus, transcription factors such as TCF and LEF interact with c-repressor Groucho or CtBP to repress Wnt-specific target genes (Cavallo et al. 1998; Fang et al. 2006).

Upon activation by Wnt binding, LRP5–LRP6 phosphorylation and Dishevelled aggression serve as mediator and lead to the translocation of Axin to the plasma

membrane and thus inactivates the destructive complex (Bilić et al. 2007). Cytosolic β-catenin could accumulate and enter the nucleus afterward, and the mechanism of the translocation still remains unclear. In the nucleus, it forms a transcriptionally active complex with LEF and TCF transcription factors by displacing Groucho and interacting with other co-activators such as BCl9, Pygopus, CBP (CREB-binding protein), or Hyrax, promoting activation of Wnt target genes (Hecht et al. 2000; Mosimann et al. 2006) (Fig. 6.1).

In addition to canonical Wnt pathway described above, some Wnt ligands and Frizzled receptors, and the Dishevelleds, are capable of activating a β-catenin independent, non-canonical Wnt signaling cascade, including the planar cell polarity (PCP) pathway and the Ca^{2+}-dependent Wnt signaling pathway (Seifert and Mlodzik 2007; Veeman et al. 2003). They may function in regulating polarization of cells and directed cell motility, as well as transforming capacity in cell culture. However, mutations in non-canonical Wnt pathway components have not been reported to be associated with tumorigenesis.

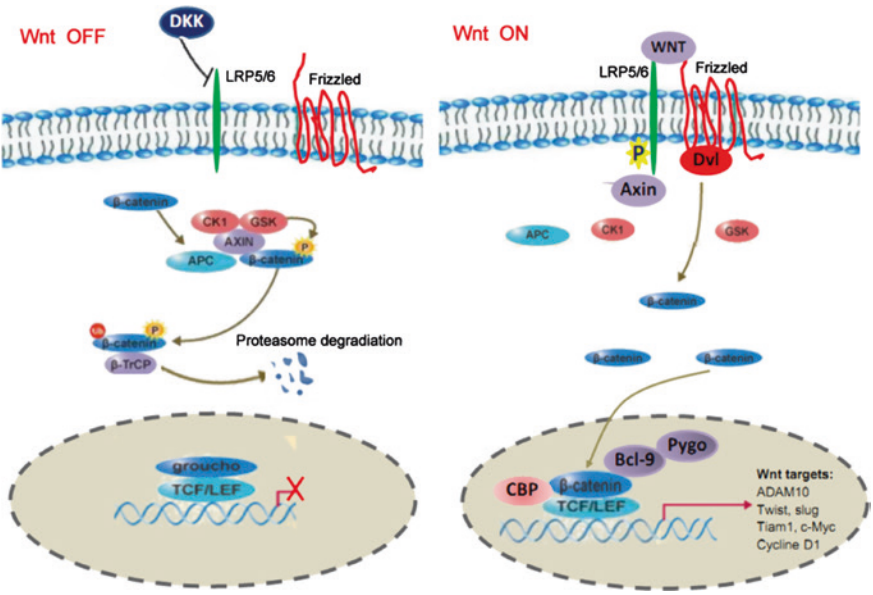

Fig. 6.1 The canonical Wnt signaling pathway. Under quiescent state, newly synthesized β-catenin binds to and is phosphorylated by a destructive complex consisting of two scaffold proteins—APC and axin—and two kinases, CKI and GSK3 (Peters et al. 1999; Amit et al. 2002). The phosphorylated β-catenin can be recognized by a b-TrCP-containing E3 ligase, which leads to proteasomal degradation of β-catenin (Liu et al. 2002; Aberle et al. 1997). Wnt binding to the receptors leads to inactivation of the destructive complex (Bilić et al. 2007). Cytosolic β-catenin then accumulates and enters the nucleus. In the nucleus, β-catenin forms a transcriptionally active complex with LEF and TCF transcription factors and other co-activators such as BCl9, Pygopus, CBP (CREB-binding protein), or Hyrax to displace Groucho and promote activation of Wnt target genes (Hecht et al. 2000; Mosimann et al. 2006)

Decades of studies on model organisms have revealed that a variety of stem cells are regulated by the Wnt signaling pathway. In vitro-cultured mouse ESCs were laid on a layer of fibroblasts in order to obtain the necessary factors, among which Wnt signal was found to be necessary for mESC self-renewal and pluripotency maintenance (Young 2011). Other approaches that activate this pathway are also effective, including overexpression of an active form of β-catenin or treatment with GSK3 inhibitors (Sato et al. 2003). However, in terms of human ESC, whether Wnt/β-catenin signaling maintains them in an undifferentiated and self-renewing state, or whether it promotes differentiation, remains controversial (Day et al. 2005). During the process of gastrulation, Wnt pathway is required for generation of primitive streak (PS), which will ultimately differentiate into mesoderm or definitive endoderm, whereas ectoderm formation is blocked by this pathway (Lindsley et al. 2006; Aubert et al. 2002). Further differentiation after germ layer formation is also regulated by Wnt/β-catenin signaling; for example, it inhibits cardiac differentiation and may redirect the cells to alternate mesodermal fates like hematopoietic lineage (Murry and Keller 2008; Trompouki et al. 2011). Reports also show that non-canonical Wnt/PCP and Wnt/β-catenin signaling cooperates to regulate the cell-fate choice in asymmetrically dividing cells in Xenopus embryo by restricting Lrp6 to the basolateral part of the stem cell (Glinka et al. 2011).

During adulthood, Wnt/β-catenin pathway also regulates proliferation and differentiation of adult stem cells, among which the best well characterized might be ISC. In mammalian small intestine, ISCs are located in the bottom of the crypt, while their progenies move upward to the villi. Paneth cells surrounding ISCs secrete WNT3 to maintain ISCs via induced expression of Wnt target genes such as *cMyc* and *cyclin D* (Li and Xie 2005). Other targets of β-catenin/Tcf signaling include ephrin receptors EphB2 and EphB3, which function in establishing crypt–villus boundaries and positioning of paneth cells at the crypt bottom (Batlle et al. 2002). Besides, additional Wnt signals could promote differentiation of paneth cells by the activation of genes specific to paneth cells, such as *cryptidin* (van Es et al. 2005).

Compared to its mammalian counterparts, *Drosophila* midgut provides a simpler model to study ISC regulation (Micchelli and Perrimon 2005). Along the midgut, Wg ligand secreted by the underlying muscle cells contributes to self-renewal of ISCs to maintain the stem cell pool (Lin et al. 2008). During tissue regeneration, Wg could also be induced in progenitors to promote ISC proliferation (Cordero et al. 2012). Hair follicle stem cells are also regulated by the Wnt/β-catenin signaling pathway. In comparison with ISCs, bulge stem cells remain quiescent when undergoing a resting phase and reside in a Wnt-restricted environment (DasGupta and Fuchs 1999). TCF3 with no β-catenin association may function to maintain skin stem cells in an undifferentiated state through repression of specific TCF target genes. Once entering regenerative phase, Wnt signaling pathway is activated and β-catenin stabilized, which will relieve TCF3 repression. Meanwhile, β-catenin interacts with another LEF/TCF member LEF1, which will activate bulge stem cells and generate new hair follicles (Lowry et al. 2005). As the stem cells proliferate and undergo a differentiated lineage, β-catenin binds to

other TFs to promote hair shaft cell differentiation, while β-catenin deficiency leads to the genesis of epidermal cell, indicating that Wnt signaling also participates in fate choice (Lowry et al. 2005). These studies indicate that by binding to different partners, Wnt/β-catenin pathway could coordinately regulate self-renewal, proliferation as well as terminal differentiation of hair follicle stem cells (Blanpain et al. 2007; Choi et al. 2013).

HSC residing in bone marrow is the origin of blood cells and immune cells. Wnt ligands, secreted by HSCs themselves as well as by the microenvironment, are responsible for self-renewal of HSC and progenitor cells, as well as maintaining them in an undifferentiated state (Rattis et al. 2004). Deletion of β-catenin in mouse models blocks long-term growth and maintenance of HSC and reduced possibility to develop BCRABL-induced chronic myelogenous leukemia (Austin et al. 1997; Jamieson et al. 2004).

In the nervous system, both central and peripheral, astrocytes generate Wnt3a to promote proliferation and neuronal fate commitment of neural precursors via canonical Wnt/β-catenin pathway (Lee et al. 2004). Wnt also promotes differentiation of NSCs into neuronal and astrocyte lineages in a time- and location-dependent manner (Toledo et al. 2008). In other systems, including mammary stem cells (MaSC) and airway stem cells, canonical Wnt pathway regulates their maintenance and self-renewal in a manner similar to that described above (Zeng and Nusse 2010; Zhang et al. 2008).

The common requirement in various types of stem cells makes the Wnt signaling cascade critical in both organ development and tumorigenesis. Depletion of Wnt/β-catenin activities will result in a series of morphological and functional defects, including the absence of intestinal crypts or hair follicles (Pinto et al. 2003; Andl et al. 2002). On the contrary, aberrant activation of Wnt pathway will lead to hyper-accumulation of stem and progenitor cells, which further might induce tumorigenesis. Leukemia, breast cancer, and the majority of familial and sporadic colon tumors are, to some extent, associated with mutations that lead to constant activation of Wnt signaling pathway (Jamieson et al. 2004; Korkaya et al. 2009; Phelps et al. 2009).

6.3 Notch Pathway

The first Notch mutant was identified in *Drosophila* a century ago by Morgan (1917). The Notch pathway is evolutionarily conserved from invertebrates to vertebrates and plays fundamental roles in a broad range of development processes.

In the canonical Notch pathway of *Drosophila*, the receptor Notch, a single-pass transmembrane receptor consisting of a large extracellular region with epidermal growth factor-like repeats and an intracellular region, interacts with the membrane-bound ligands Delta(Dl) or Serrate(Ser) (Delta-like and Jagged in mammalian) from the neighboring cells. A cascade of proteolytic events is triggered after the ligand–receptor interaction, resulting in the release of the

intracellular domain (NICD) of Notch to the cytoplasm. The NICD then translocates into the nucleus to form a transcriptional complex with the co-activator Mastermind (Mam) and the DNA-binding protein Suppressor of Hairless [Su(H)] (CSL, CBF1/RBPJK in mammalian) to regulate gene expression (Bray 2006; Kopan and Ilagan 2009) (Fig. 6.2). There is also increasing evidence for a ligand- or transcription-independent non-canonical Notch pathway that exerts important biological functions, which we will not discuss here but have been reviewed elsewhere by Heitzler (2010), Andersen et al. (2012).

Notch signaling has been implicated to function critically in many kinds of stem cell lineages, such as stem cells in skin, nervous system, hematopoietic system, muscle, liver, kidney, and intestine. As mentioned above, Notch signaling is a very simple signaling cascade even without a second messenger, but its function is highly context dependent and can be modulated at multiple levels, allowing it to play a variety of biological roles from stem cell maintenance to multiple cell lineage differentiation. Here, we mainly focus on its roles in embryonic stem cells (ESCs) and adult stem cells in hematopoietic system and intestine.

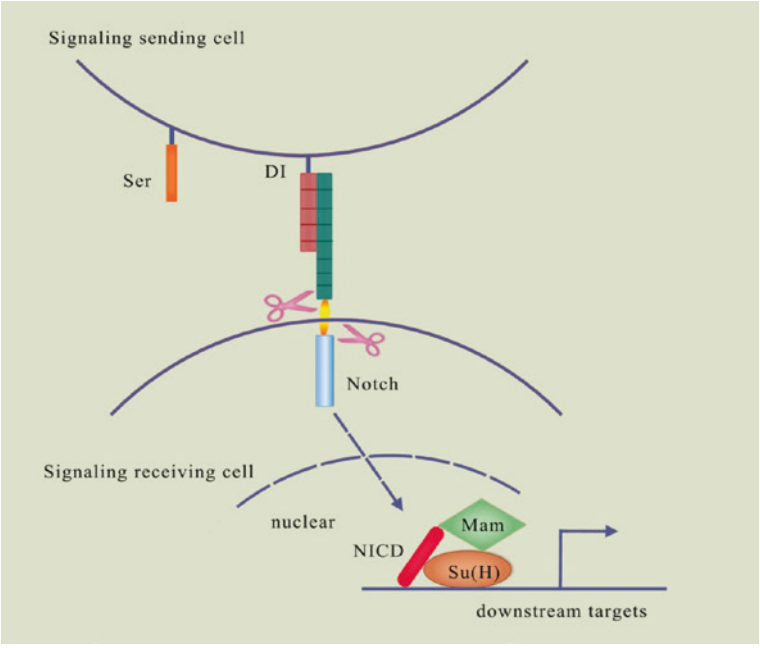

Fig. 6.2 The canonical Notch signaling pathway in *Drosophila*. The single-pass transmembrane receptor Notch can be activated by the membrane-bound ligands, Delta (*Dl*) or Serrate (*Ser*), from neighboring signal-sending cells. The ligand–receptor interaction induces a cascade of proteolytic events, which leads to the release of Notch intracellular domain (*NICD*) from the cell membrane. The NICD translocates into the nucleus, where it forms a transcriptional complex with the co-activator Mastermind and the DNA-binding protein Suppressor of Hairless (Su(H)) to regulate its target gene expression

There is no detectable Notch activity in human embryonic stem cells (hESCs), and Notch is not required for the hESC maintenance (Noggle et al. 2006). But Notch plays important roles in cell-fate decision during ESC differentiation. Activation of Notch signaling in ESCs under differentiation condition promotes the neural commitment of ESCs, resulting in differentiation into neuroectodermal progenitor cells (Lowell et al. 2006). Conversely, loss or downregulation of the Notch activity leads to cardiac mesodermal differentiation (Schroeder et al. 2003; Nemir et al. 2006; Jang et al. 2008). Therefore, Notch is not required for ESC maintenance but modulates the outcome during differentiation.

The hematopoietic system is relatively complex, which consists of multiple cell lineages. Notch has involved in many aspects of hematopoiesis. Notch signaling is required for the generation of HSCs in the aorta-gonad-mesonephros (AGM) region during embryogenesis and in the long-term definitive hematopoiesis past the early fetal liver stage (Kumano et al. 2003; Robert-Moreno et al. 2008; Hadland et al. 2004). It also functions in HSC maintenance in the marrow. Many Notch ligands are expressed by the hematopoietic niche (Karanu et al. 2001; Fernandez et al. 2008), and increased Notch activity in HSCs promotes expansion of HSCs and hematopoietic progenitor cells in vivo and in vitro (Calvi et al. 2003; Butler et al. 2010; Stier et al. 2002; Varnum-Finney et al. 2003; Karanu et al. 2000). In contrast, inhibition of Notch activity in HSCs displays no detectable effect on HSC maintenance (Mancini et al. 2005; Maillard et al. 2008). Therefore, Notch signaling is dispensable for adult HSC maintenance, but its activation is sufficient to promote HSC proliferation. In addition, Notch signaling is also essential for the differentiation of HSCs and other hematopoietic progenitor cells. Enforced Notch activity in the hematopoietic progenitor cells promotes T cell commitment (de La Coste et al. 2005; Radtke et al. 2004), and loss of the activity leads to T cell deficiency (Radtke et al. 1999). Notch also plays a role in cell-fate decision among myeloid progenitors, and activation of Notch both in vivo and in vitro induces megakaryocyte development (Mercher et al. 2008). Therefore, the role of Notch signaling in hematogenesis is important for lineage commitment at multiple branch points of hematopoiesis. Further investigation into the mechanism of the Notch function in different lineages may facilitate the understanding of the complexity of Notch function in hematopoietic system.

Compared to the mammalian system, the *Drosophila* midgut is a much simpler model system for studying signaling regulation of stem cells. The midgut ISCs usually asymmetrically divide into a new ISC and an intermediate enteroblast (EB), and EB can further differentiate into either an enterocyte (EC) or an enteroendocrine (ee) cell (Micchelli and Perrimon 2005; Ohlstein and Spradling 2005, 2007). Notch signaling plays crucial roles in the binary cell-fate decision and terminal differentiation of EB. The Notch ligand Dl is specifically expressed in ISCs and activates Notch activity in their immediate daughter EBs. Overexpression of the NICD in progenitor cells invariably induces EC differentiation, while loss of Notch leads to the expansion of ISC-like cells and ee cells. Therefore, it has been proposed that EBs that receive high levels of Notch activity will differentiate into ECs, whereas EBs that receive low or no Notch activity will differentiate into ee

cells (Micchelli and Perrimon 2005; Ohlstein and Spradling 2005, 2007). In mammalian intestine, Notch regulates both the maintenance and differentiation of ISC. In contrast to that in *Drosophila* gut, ISCs in mammals are also signal-receiving cells where the receptors Notch1 and Notch2 are expressed (Fre et al. 2011; Pellegrinet et al. 2011). Activation of Notch in ISCs promotes their amplification, and activation of Notch in progenitor cells favors absorptive cell differentiation over secretory cell differentiation (Fre et al. 2005; Ueo et al. 2012). Conversely, loss of Notch activity leads to the loss of ISCs and increased production of the secretory goblet cells (Ueo et al. 2012; Riccio et al. 2008; Milano et al. 2004). In addition to HSCs and ISCs, Notch is involved in the regulation of many other types of stem cells, which will not be discussed here but are summarized by Liu and Carolina (Liu et al. 2010; Perdigoto and Bardin 2013).

Taken together, Notch signaling is involved in many aspects of stem cell behavior, including maintenance, cell-fate decision, and terminal differentiation. Immediate questions remaining to be answered include how the specificity of Notch function in a different context is achieved and how the pathway is regulated to meet the needs during tissue homeostasis and in response to environmental changes.

6.4 EGFR Pathway

As a central element for a variety of cellular response and signaling transduction network, the epidermal growth factor receptor (EGFR) family has fundamental roles in the development of multicellular organisms. EGFR signaling pathway has been reported to regulate many cellular functions including the cell survival, motility, proliferation, and cell-fate decision. Disruption of the signaling pathway is frequently implicated in the development of human tumors, proposing EGFR as a prognostic marker or target in cancer therapy. In this part, we will focus on the critical role of EGFR signaling pathway as regulators of stem cell properties including maintenance and differentiation.

The role of EGFR in regulating stem cell proliferation and self-renewal has been well described in many tissues, including neural system, intestine, and mammalian epidermis.

Proliferation of stem cells is crucial for tissue homeostasis and regeneration during wound repairing, especially in tissues with high turnover. A typical system is *Drosophila* intestine, where EGFR promotes the proliferative capacity of ISCs to maintain gut homeostasis and regeneration after damage (Jiang et al. 2011). The EGFR ligand, Vein, is specifically expressed in visceral muscle surrounding the midgut epithelium as a proliferating niche signal. And two additional EGFR ligands, Spitz and Keren, serve as autocrine signals to redundantly promote ISC proliferation and maintenance (Biteau and Jasper 2011; Xu et al. 2011). Damage in midgut epithelium induces multiple EGFR ligands to activate EGFR/Ras/MAPK signaling pathway in ISCs, which is required in ISC

proliferation and tissue regeneration (Jiang et al. 2011). The synergetic cooperation of EGFR with other signaling pathways, including JAK/STAT and Wingless signaling, is essential for ISC maintenance (Jiang et al. 2011; Xu et al. 2011; Buchon et al. 2010).

Similar in *Drosophila* intestine, interaction of EGFR with other signaling pathways is also crucial in the regulation of stem cell maintenance in nervous system. In the adult brain, the subventricular zone (SVZ) and the dentate gyrus are the niches that maintain neural stem cells (NSCs) and neural progenitor cells (NPCs), and balance of sizes between these two populations is critical for brain homeostasis (Alvarez-Buylla and Lim 2004). In the SVZ, Notch signaling is required to maintain NSCs, while EGFR is responsible for the development of NPCs (Hitoshi et al. 2002; Alexson et al. 2006; Lillien and Raphael 2000). Through direct interaction between NSCs and NPCs, the cooperation of EGFR signaling and Notch signaling occurs to maintain the balance between these two populations (Aguirre et al. 2010).

Along with its well-described role in regulating cell survival and proliferation, EGFR signaling also functions in the differentiation of certain types of stem cells or progenitor cells. In *Drosophila* eye disc, EGFR activation triggers differentiation of all retinal cell types (Freeman 1996). The generation of distinct cell fates depends on the combinatorial effect of EGFR signaling with other signal responses (Flores et al. 2000). Combination of EGFR and Notch signaling regulates cone cell specification, while specification of R7 cells requires the Sevenless receptor tyrosine kinase besides EGFR and Notch signaling (Flores et al. 2000; Cooper and Bray 2000). In other species like freshwater planarians, EGFR signaling is also essential in the process of differentiation and morphogenesis. Silencing planarian EGFR gene using RNAi results in the abnormal differentiation of certain cell types and various tissue defects (Fraguas et al. 2011).

EGFR also regulates the mobility of stem cells. In various tissues, EGFR signaling is linked to a more mobile phenotype in both immature progenitors and committed cells. In transplanted embryonic progenitor cells, continuous EGF signaling stimulates their proliferation and migration (Fricker-Gates et al. 2000). In the case of mature tissue system, active EGFR signaling also enhances the migration of NSCs and HSCs, providing a possible pharmacological strategy for cellular transplantation in disease therapy (Ryan et al. 2010; Boockvar et al. 2003; Ayuso-Sacido et al. 2010).

EGFR signaling pathway plays a central role in a variety of fundamental cellular functions including cell growth, proliferation, transformation, and mobilization. The ability that the single receptors function in such diverse processes attributes to the cell types that receive the EGF stimulation and combinatorial effect of multiple signaling pathways. Due to the complexity of the EGFR transduction network, the mechanism regulating the interconnected network and the resulting responses still remains incompletely clear. Since EGFR signaling is frequently implicated in hyper-proliferative diseases, more mechanistic studies of its involvement in cellular responses under both normal and pathological conditions are needed for the understanding of disease mechanisms and the development of therapies.

6.5 BMP Pathway

BMP signaling has diverse functions in multicellular organism development and recently has been reported to function as an essential regulator of stem cell maintenance and cell-fate decision. In this section, we will discuss how BMP signaling regulates stem cell properties and its potential role in cancer development.

BMPs belong to TGF-β superfamily and signal through receptor-mediated intercellular pathway to regulate expression of target genes. There are two types of receptors, one with type I receptor (Bmpr II) and three with type II receptors (Alk2, Alk3, and Alk6). Activation of the BMP pathway involves the formation of heteromeric complex of type I receptor and type II receptor upon ligand binding, which mediates the phosphorylation of type I receptor. Then, downstream intercellular messengers are three classes of Smad proteins: receptor-mediated Smad1/5/8 (R-Smad), the common mediator Smad4 (Co-Smad), and the inhibitory Smad6/7 (I-Smad). Activated type I receptor mediates R-Smad phosphorylation, which induces the formation of R-Smad/Co-Smad complex. The heteromeric Smad complex then translocates to the nucleus and regulates target gene expression in cooperation with other transcription factors. I-Smad functions to negatively regulate the Smad signaling pathway. A downstream pathway in parallel with the canonical BMP pathway is TAK1/MAPK pathway. TAK1 is a MAPKKK tyrosine kinase, which is activated by linking to the receptor mediated by X-linked inhibitor of apoptosis (XIAP). Notably, TAK1 also participates in JNK and NF-kB pathway, providing a possible means of cross talk between BMP and other signaling pathways.

The BMP pathway has a role in maintaining mammalian embryonic stem cell self-renewal. ESCs are stem cells with widest developmental capacity, which can contribute to all three germ layers: the ectoderm, mesoderm, and endoderm (Chambers and Smith 2004). ESCs are derived from the inner cell mass (ICM) of the blastocyst embryos and can be stably maintained in in vitro culture under a proper condition, thus providing a widely used system to study self-renewal and commitment of stem cells.

A series of studies in mouse ES cells have shown that BMP pathway is able to maintain cultured mES cells in undifferentiated state (Ying et al. 2003). mESCs can be cultured with a layer of mouse embryonic fibroblast (MEF) cells to produce supporting factors including leukemia inhibitory factor (LIF). LIF effectively supports mESCs' self-renewal in culture conditions containing serum, but in the absence of feeder cells or serum, LIF alone cannot maintain pluripotency but induce neural differentiation of mESCs (Ying et al. 2003; Ying and Smith 2003). However, treatment in combination with LIF and BMP4 suppressed neural differentiation and is sufficient to maintain pluripotency of mESCs without feeder cells or serum (Ying et al. 2003). It is well known that in contrast to LIF, which favors neural differentiation through Stat3, BMP signaling inhibits neural differentiation (Ying et al. 2003; Tropepe et al. 2001). Therefore, coordination of LIF and BMP signaling and their balanced mutual inhibition are crucial for mESC maintenance.

By contrast, BMPs in human ESCs promote differentiation (Pera et al. 2004). Unlike mESCs, hESCs need basic fibroblast factors (bFGF) rather than LIF to support self-renewal and pluripotency when cultured with a feeder layer or fibroblast-conditioned media. High level of BMP signaling was found in unconditionally cultured hESCs. Moreover, in the absence of feeder layer or conditioned media but in the presence of bFGF, cultured hESCs require exogenous BMP antagonist Noggin to maintain pluripotency (Wang et al. 2005; Xu et al. 2005). This divergent response to BMP signaling may be due to the fundamental distinctions between mESCs and hESCs, as they are probably at different pluripotent states (Pera and Trounson 2004).

BMP signaling is also one of the key regulators of cell-fate commitment in stem cell differentiation. Here, we take neural crest stem cells (NCSCs) as an example. In vertebrates, the neural crest originates from dorsal neural tube during early development and will later migrate and generate multiple cell types including melanocytes, smooth muscle, and neurons and glia of peripheral neural system. Among the neural crest are subsets of pluripotent NCSCs, which can self-renew and differentiate into diverse cell types. In rat NCSCs, BMPs can induce their differentiation into autonomic precursor cells expressing Mash1, while continuous BMP signaling contributes to neuronal commitment (Shah et al. 1996). Therefore, cell-fate determination directed by BMPs is a multistep process.

The BMP pathway has a central role in the maintenance of GSCs in *Drosophila*. GSCs and their surrounding niche cells are located at the germarium in *Drosophila* ovary. The cap cells directly contacting GSCs, together with the filament cells, form the supporting niche for GSCs (Lin 2002; Xie and Spradling 2000). BMPs, including Dpp and Gbb, are key molecules that are secreted by cap cells in GSC maintenance and self-renewal. Dpp/Gbb signaling promotes GSC expansion and disruption of their expression resulting in GSC loss (Lin 2002; Xie and Spradling 2000). Inhibition of the differentiation-promoting gene, bag of marbles (bam), is critical for Dpp/Gbb to regulate GSC self-renewal (Chen and McKearin 2003; Song et al. 2004). This machinery also functions in maintaining GSCs in *Drosophila* testis, where hub cell plays the role of niche to support GSCs. But in testis, other pathways including JAK/STAT signaling are also essential for GSC maintenance (Kiger et al. 2001; Tulina and Matunis 2001).

In summary, BMP signaling pathway has diverse functions in different organisms at different developmental stages. Much evidence has shown that BMP signaling plays an essential role in promoting self-renewal of many types of stem cells, such as mammalian ESC, *Drosophila* GSC and various somatic stem cells. BMP signaling is also involved in the regulation of cell-fate determination, which is well studied in the development of NCSCs. In most cases, BMP functions in coordination with other signaling pathways to regulate stem cell properties. Therefore, balanced control of stem cell activity involves delicate mechanisms, which usually requires the cooperation of multiple signaling pathways.

6.6 JAK-STAT Pathway

The Janus kinase/signal transducers and activators of transcription (JAK-STAT) pathway, initially discovered in mammalian about two decades ago (Firmbach-Kraft et al. 1990; Wilks et al. 1991; Shuai et al. 1993; Muller et al. 1993; Watling et al. 1993; Shuai et al. 1993), are conserved between *Drosophila* and mammals. Subsequent studies in *Drosophila* and mammals have uncovered its role in regulating diverse biological processes.

The JAK-STAT pathway consists of three main components: the receptors, JAKs, and STATs. The two JAKs, which are associated with the closed receptor, are brought together after ligand–receptor binding and phosphorylate each other. The phosphorylated JAK can recruit and phosphorylate STAT in the cytoplasm. Then, two phosphorylated STATs dimerize and translocate into the nucleus and bind to DNA to promote transcription. The JAK-STAT signaling pathway can be activated by several kinds of ligands, such as interferons, interleukins, and growth factors. It can also be regulated by multiple modulators at several levels. For example, protein phosphatases can inhibit active JAK by removing its phosphates, and protein inhibitors of activated STAT (PIAS) can prevent binding of active STAT to DNA (Ungureanu et al. 2003, 2005; Rakesh and Agrawal 2005).

A role for the JAK-STAT pathway in stem cell maintenance was first implicated in *Drosophila* germline stem cell niche (Tulina and Matunis 2001; Kiger et al. 2001), and now, it has become clear that it plays important roles in a variety of stem cells. The JAK-STAT pathway plays an important role in the maintenance of ESC in mouse and swine and in facilitating the reprogramming (Onishi et al. 2012; Wu et al. 2014; Tang et al. 2012; Tang and Tian 2013; Ernst et al. 1999; Hao et al. 2006). But its function between mouse and human is a little controversial (Dreesen and Brivanlou 2007; Humphrey et al. 2004). Here, we mainly review its role in regulating tissue-specific stem cells and cancers.

In the *Drosophila* testis, the JAK/STAT signaling ligand Upd is specifically secreted from the hub, the niche of the GSCs, and somatic cyst stem cells (CySCs), which activates the JAK-STAT signaling pathway in GSCs and CySCs (Tulina and Matunis 2001; Kiger et al. 2001). JAK-STAT activity in CySCs is sufficient to induce self-renewal of both GSCs and CySCs, while its activity in GSCs is required for DE-cadherin-mediated attachment of GSCs to the hub (Leatherman and DiNardo 2008, 2010). There is evidence that STAT3 can regulate spermatogonial stem cell differentiation in mouse (Oatley et al. 2010). In addition, the differentiated GSC induced by the depletion of JAK-STAT signal can undergo dedifferentiation when the JAK-STAT signal is restored to its normal level (Brawley and Matunis 2004).

Similar to the stem cells in testis, the *Drosophila* prohemocyte progenitor cells are supported by a niche named posterior signaling center (PSC). Signals from PSC lead to JAK-STAT activation in the prohemocytes for prohemocyte maintenance (Krzemień et al. 2007). But unlike in testis, JAK-STAT activation in prohemocytes is not likely induced by Upd, and other signals from PSC are needed

(Makki et al. 2010). JAK-STAT signaling is also required for hematocyte–lamellocyte transition after infestation (Stofanko et al. 2010). The JAK-STAT pathway also functions in hematopoietic stem cell maintenance and differentiation in mammalian (Bradley et al. 2004; Snow et al. 2002; Kato et al. 2005; Wang et al. 2009). For example, the loss of STAT5 activity in mice leads to the deficiency of multipotent hematopoietic progenitors and consequently several hematopoietic cell lineages, which causes inefficient repopulation upon irradiation (Bradley et al. 2004; Snow et al. 2002). The mechanism of JAK-STAT regulation in mammalian hematopoiesis is rather complex and is extensively reviewed by Stine and Matunis (2013).

Together with other signaling pathways, the JAK-STAT pathway regulates both proliferation of ISCs and differentiation of intestinal EBs to maintain homeostasis of the fly midgut. Loss of the JAK-STAT activity in ISCs decreases the proliferation rate of ISC and vice versa (Beebe et al. 2010; Lin et al. 2010; Liu et al. 2010). JAK-STAT activity is also essential for EB differentiation, as mutation in the pathway blocks the progenitor cell differentiation at EB stage (Beebe et al. 2010; Lin et al. 2010). The JAK-STAT pathway can also induce rapid proliferation and differentiation of the progenitor cells in response to stress or bacterial infection (Jiang et al. 2009; Buchon et al. 2009).

The JAK-STAT signaling pathway is important in controlling the balance between stem cell self-renewal and differentiation in *Drosophila* neural system. It is required for neuroepithelial (NE) stem cell maintenance, and the loss of JAK-STAT activity leads to the loss of NE due to precocious differentiation (Yasugi et al. 2008; Ngo et al. 2010; Wang et al. 2011). The JAK-STAT pathway also plays important roles during neurogenesis in mammals to ensure the appropriate generation of the right cell type at different developmental stages of the neural system. For example, the elevated JAK-STAT activity is required for neurogenesis–gliogenesis transition (Barnabé-Heider et al. 2005; He et al. 2005).

The JAK-STAT pathway may also function in cancer stem cells (CSCs). The upregulation of JAK-STAT signaling activity has been found in many CSCs (Zhou et al. 2007; Birnie et al. 2008; Cook et al. 2014), and the activity is required for the maintenance of CSCs (Zhou et al. 2007; Cook et al. 2014; Sherry et al. 2009). Consistent with its role in CSCs, many inhibitors that target the JAK-STAT pathway could be useful in the treatment of certain types of cancers (Hart et al. 2011; Pardanani et al. 2013; Harrison et al. 2012; Verstovsek et al. 2012; Mascarenhas et al. 2014).

In conclusion, the JAK-STAT pathway plays an important role in regulating tissue homeostasis by regulating the balance between stem cell self-renewal and differentiation. It usually runs in parallel or interacts with many other signaling pathways to control the stem cell maintenance and differentiation. Many studies of the JAK-STAT pathway have been done in *Drosophila* because of less redundancy and complexity of this pathway. More investigations are needed in mammals to determine whether the lessons learned from *Drosophila* can be applied to mammalian stem cells and to study its relationship with other signaling pathways in the regulation of tissue stem cells and cancer.

6.7 Hedgehog Pathway

The Hedgehog (Hh) family proteins are key morphogens that direct cell patterning of embryonic tissues and tissue homeostasis throughout animal development. The Hh signaling pathway regulates diverse cellular responses including cell survival, proliferation, and fate determination. Disruption of Hh signaling is often involved in developmental disorders and tumorigenesis. Here, we will discuss the role of Hh signaling in regulating stem cell maintenance.

The *Hh* gene was first identified in *Drosophila*. Vertebrate Hh counterparts, including the Desert Hedgehog (Dhh), Indian Hedgehog (Ihh), and Sonic Hedgehog (Shh), were found shortly thereafter, and the developmental function of Hh molecules is evolutionarily conserved in *Drosophila* and vertebrates. Moreover, main components of Hh signal transduction pathway are also evolutionarily conserved. In both *Drosophila* and mammals, ligand-free Patched (Ptc) protein restrains the activation of the transmembrane protein Smoothened (Smo) by triggering Smo degradation and blocking membrane localization of Smo. Binding of Hh blocks Ptc activity and liberates Smo to translocate to the membrane. The downstream intercellular cascade resulting from Smo activation ended with the translocation of Ci/Gli family into nucleus to direct the expression of target genes. Significant differences still exist in the pathway components between invertebrates and mammals, especially the transduction machinery from the receptor to the Ci/Gli transcription factors.

In addition to its well-established function as developmental morphogen, Hh signaling also regulates stem cell self-renewal and tissue homeostasis. In mammals, the expression of several stem cell-related genes, including genes encoding MYC, BMI1, Cyclin D1, Nanog, and insulin-like growth factor 2 (IGF2), is promoted by Hh signaling (Davidson et al. 2012; Briscoe and Therond 2013).

Hh is required for the maintenance of stem cells in a variety of adult tissues. In neural system, blockage of Hh signaling by inhibiting Smo decreases the proliferative capacity of neural stem cell both in vitro and in vivo (Machold et al. 2003; Lai et al. 2003). In the case of HSC, treatment of Shh increases the expansion of HSCs in vitro and in vivo (Bhardwaj et al. 2001). Similar phenomena are found in *Drosophila* ovary system, where Hh is a major factor controlling proliferation and maintenance of ovary somatic stem cells (Zhang and Kalderon 2001).

Consistent with its crucial role in controlling cell patterning and stem cell self-renewal, Hh is implicated in the regeneration and damage repair of a variety of tissues. In newt lens and limb, the Hh proteins are often expressed in the injury-induced dedifferentiated cells, and tissue regeneration is blocked by treatment with cyclopamine, a specific inhibitor of Hh pathway (Tsonis et al. 2004; Imokawa and Yoshizato 1997; Roy and Gardiner 2002). Blocking Hh pathway by cyclopamine also disrupts tissue repair in other species, such as the fin of zebrafish and the facial nerves of mouse (Laforest et al. 1998; Akazawa et al. 2004).

However, Hh families do not always function to promote cell proliferation during tissue regeneration. Different tissue types within an organ system may

have different responses to a single signaling molecule, depending on the type of responding cells and the coordination of multiple signaling pathways. A good example is the Hh signaling network in gastrointestinal tract, where epithelial proliferation is regulated by multiple signaling pathways including Hh signaling and Wnt signaling. The proliferation of epithelium in the esophagus, stomach, and pancreas is promoted by Hh signaling, but proliferation in the intestine is suppressed by Hh signaling through its negative effects on Wnt signaling pathway (Katoh and Katoh 2006).

In summary, Hh is an evolutionarily conserved signaling pathway that has various and critical functions in the process of embryonic morphogenesis and adult tissue homeostasis. Diverse cellular responses, including cell survival, growth, proliferation, and cell-fate specification, are regulated by Hh signaling pathway network. Moreover, Hh signaling pathway has been implicated in various human tumors, proposing Hh signaling components as potential targets in cancer therapy. However, because of its complexity, the machinery of Hh is not completely understood yet. Therefore, many questions remain to be studied in exploring the details of Hh signaling network as well as the mechanisms of this process in regulating tumor progression for developing therapeutic strategies.

6.8 Hippo Pathway

The Hippo pathway is a newly characterized, evolutionarily conserved signaling cascade. The first component of this pathway was first identified by Wan Yu's and Peter J. Bryant's group in 1995 through mosaic clonal screens for genes involved in tissue growth control in *Drosophila* (Xu et al. 1995; Justice et al. 1995). The mutation in this pathway leads to increased organ size through increased cell proliferation and decreased cell death. The number and the activity of the stem cells in organs may play an important role for the organ size (Stanger et al. 2007). Therefore, the Hippo pathway may control the organ size by regulating the tissue stem cells (Camargo et al. 2007; Song et al. 2010; Lee et al. 2010; Jansson and Larsson 2012; Nejigane et al. 2013).

The core components of this pathway have been well characterized (Xu et al. 1995; Justice et al. 1995; Tapon et al. 2002; Wu et al. 2003; Udan et al. 2003; Harvey et al. 2003; Jia et al. 2003; Pantalacci et al. 2003; Dong et al. 2007), which consists of a highly conserved kinase cascade (Ste20-like kinase Hippo and NDR family kinase Warts in *Drosophila*, MST1/2 and LATS1/2 in mammalian) and downstream transcription co-activators (Yorkie in *Drosophila*, YAP/TAZ in mammals). In *Drosophila*, Hippo (Hpo) forms an active complex with the scaffolding protein Salvador (Sav) to directly phosphorylate and activate Warts (Wts) and its regulatory protein Mob (Tapon et al. 2002; Lai et al. 2005). Then, the active Wts/Mob complex phosphorylates the Yorkie (Yki) to promote its binding to 14-3-3 protein, which inhibits the translocation of Yki into the nucleus, where it act as the co-activator for the TEAD/TEF family transcription factor Scalloped (Sd) to

promote gene expression, thereby facilitating cell proliferation and survival (Dong et al. 2007; Vassilev et al. 2001; Huang et al. 2005; Mahoney et al. 2005; Oh and Irvine 2008; Ren et al. 2010; Staley and Irvine 2012). The signal transduction in mammals is similar to that in flies (Wu et al. 2003; Lai et al. 2005; Huang et al. 2005; Tao et al. 1999). Although the core components in this pathway are well characterized, its upstream regulators are not well defined. It has been shown that apical–basal polarity proteins, cellular junction proteins, and some extracellular hormones can regulate the Hippo pathway, a subject that has been comprehensively reviewed by Jung-Soon Mo (Ramos and Camargo 2012).

The Hippo pathway was firstly characterized to control organ size by inhibiting proliferation and promoting apoptosis, and the current studies show that Hippo pathway can also regulate stem cell self-renewal and has important roles in tissue regeneration (Lian et al. 2010; Varelas et al. 2008; von Gise et al. 2012; Yimlamai et al. 2014). The Hippo pathway plays a critical role in regulating ESCs, adult progenitor cells, and CSCs. The phosphorylation of LATS together with YAP can suppress TEAD4 activity in the inside cell of preimplantation mouse embryo to distinguish mouse ICM from trophectoderm (Nishioka et al. 2009). YAP is also required for mouse ESC pluripotency, directly binds to a large number of pluripotency-related genes, and enhances reprogramming efficiency of mouse iPSs (Lian et al. 2010). TAZ is required for maintaining pluripotent gene expression in human ESC, and knockdown of TAZ results in differentiation (Varelas et al. 2008). YAP and TEAD2 are activated by LIF in mouse ESCs and are downregulated during differentiation (Tamm et al. 2011). In addition to ESC, the Hippo pathway also functions in tissue-specific progenitor cells. YAP activation in postnatal liver leads to dramatic increase in the liver size (Camargo et al. 2007). Mst1/2 mutation also results in liver overgrowth (Song et al. 2010). More recently, the Hippo pathway has been demonstrated to be essential for the maintenance of the differentiated state of hepatocyte. Its inactivation in vivo is sufficient for hepatocytes to dedifferentiate into progenitors (Yimlamai et al. 2014). The intestinal epithelium is also regulated by the Hippo pathway, and YAP overexpression results in the expansion of the intestinal progenitor cells (Zhou et al. 2011). Similarly, YAP activation in the skin leads to skin hyperplasia, which is driven by the excessive proliferation of the interfollicular stem cells (Schlegelmilch et al. 2011). But the overgrowth of the heart induced by Sav1 knockout results from the expansion of the cardiomyocytes, not the cardiac progenitors (Heallen et al. 2011), and TAZ overexpression leads to the myogenic differentiation (Jeong et al. 2010). In the nervous tissues, YAP co-localizes with neural progenitor maker Sox2, while activation of YAP or inactivation of MST1/2 results in neural progenitor expansion (Cao et al. 2008; Gee et al. 2011). More information about functions of the Hippo pathway in tissue-specific progenitor cells has been summarized (Ramos and Camargo 2012) elsewhere (Ramos and Camargo 2012). Abnormal activity of Hippo signaling has also implicated in various cancers. TAZ is essential for the self-renewal of breast CSCs and tumor progression (Cordenonsi et al. 2011). Moreover, upregulation of YAP1 acts as a determinant for maintaining esophageal CSC properties (Song et al. 2014). TAZ and YAP are also highly expressed and activated in a variety of

human cancers (Liang et al. 2014; Perra et al. 2014; Steinhardt et al. 2008; Yue et al. 2014).

The role of the Hippo signaling pathway in stem cell regulation stimulates a new line of research in cancer- and degeneration-related diseases. Although it is evident that YAP activation can mediate Wnt or Notch signaling pathway in some tissues (Yimlamai et al. 2014; Heallen et al. 2011; Xin et al. 2011) and LIF (Tamm et al. 2011) and TGF-β or BMP signaling pathway (Varelas et al. 2008; Alarcón et al. 2009; Beyer et al. 2013) in others, the immediate upstream regulators of the Hippo pathway and the mechanisms that turn on and off the pathway are still not well understood. How the Hippo pathway integrates the inputs from these multiple signals to generate the correct outputs for context-dependent function? What are the target genes that drive the appropriate cellular response? In addition, the Hippo pathway could regulate stem cell expansion and tumorigenesis through different mechanisms, and loss of different components in this pathway sometimes leads to diverse phenotypes. Therefore, further studies are needed to fully elucidate the exact role of each component of the Hippo pathway in regulating stem cells. Answers to these questions will ultimately contribute to our understanding of tissue homeostasis control, regeneration, and tumorigenesis.

6.9 Insulin Pathway

Endocrine system plays an important role in coordinately regulating the growth of multiple organs. These systemic signals, along with short-range niche signals, can function together to regulate tissue stem cells (Gancz and Gilboa 2013).

Among these hormones, insulin is a well-characterized signal that is conserved in various organisms. After binding to insulin receptor (InR), it activates a downstream cascade that ultimately affects Forkhead Group O (Foxo) and tuberous sclerosis complex 2 (TSC2), two important nodes in metabolism and energy control. Thus, insulin pathway could serve as a link between nutrient state and stem cell activity (Grewal 2009). For example, as the most energy-consuming process in female *Drosophila*, oogenesis is tightly regulated by this pathway in multiple ways. For the regulation of GSCs, insulin binds to InR on the surface of GSCs and autonomously regulates its division. Besides, it can indirectly regulate GSC activity by regulating Notch signaling in the cap cells and DE-cadherin between cap cells and GSCs (Hsu et al. 2008; Hsu and Drummond-Barbosa 2009, 2011).

Another tissue that closely relates to food consumption and metabolism is the intestine. Either in *Drosophila* or in mice, intestine changes its size according to the abundance of food. This may attribute to the altered rate of ISC proliferation and number of ECs caused by the altered production of insulin (O'Brien et al. 2011). Locally secreted *Drosophila* insulin-like peptide 3 (DILP3) by visceral muscle cells underneath the ISCs is the major player, while systemic DILPs that originated from brain IPCs may also participate in this process. Elevated level of DILPs will lead to a switch from asymmetric to symmetric division of ISCs

and expand the pool of stem cells. Besides, in response to tissue damage in the *Drosophila* midgut, systemic DILP2 secreted by brain cells participates in promoting ISC division and consequently epithelial regeneration (Amcheslavsky et al. 2009). The *Drosophila* ISCs show declined proliferation in response to caloric restriction. By contrast, ISCs in mouse small intestine increase their proliferation and number. ISCs' closest neighbors, the paneth cells, sense the caloric restriction via mTORC1 pathway. Repression of this pathway in paneth cells promotes ISC proliferation via a secreted enzyme that generates cyclic ADP ribose (cADPR) (Yilmaz et al. 2012). However, the activity of transient amplifying cells is reduced in response to caloric restriction. Therefore, different responses to insulin signaling between ISCs and transient amplifying cells may provide a mechanism to protect stem cell population under starvation conditions and at the same time to limit the production of stem cell progenies for energy reservation.

The insulin/Tor pathway also regulates homeostasis of HSCs. Cell autonomous activation of insulin/Tor pathway disrupts quiescence of HSC and finally lead to exhaustion of stem cells due to reduced self-renewal capacity. On the other hand, insulin/Tor pathway activation in the niche supports HSC self-renewal and preservation of the stem cell population (Chen et al. 2008; Kharas et al. 2010; Kobayashi et al. 2010). In addition to insulin, other hormones have also been found to regulate stem cells. Ecdysone is one example, which plays multiple roles in orchestrating development and homeostasis of GSCs in *Drosophila* (Ables and Drummond-Barbosa 2010; König et al. 2011; Morris and Spradling 2012). This hormone functions autonomously to promote GSC maintenance and self-renewal by Nurf-dependent activation of BMP activity, which otherwise will lead to differentiation. The major source of ecdysone has been located to maturing egg chambers, whose survival is dependent on food supply (Ables and Drummond-Barbosa 2010). Via this regulatory loop, the oogenesis could be modulated based on the nutrient status.

6.10 Conclusion Remarks

Tissue stem cells are important for maintaining tissue homeostasis due to their unlimited or prolonged ability of self-renewal and their cellular multipotency. These abilities are intrinsic properties but can be maintained and regulated by signaling pathways. As reviewed above, in both invertebrate and vertebrate models, self-renewal and differentiation of stem cells are commonly regulated by local signals as well as systemic signals. The local microenvironment ensures long-term maintenance of stem cells and proper division activity and differentiation potential required for tissue homeostasis. The systemic signals usually serve to coordinate stem cell activity with tissue/organ growth and with needs, such as nutrient availability. Although many signaling pathways are frequently utilized to control stem cells, there is no common signaling circuitry that controls stemness of all or most types of tissue stem cells. Instead, each type of tissue stem cells is usually

regulated by a distinct set of signaling pathways. Some of them are critical for self-renewal and proliferation, whereas some of them are more important for the differentiation of progenitor cells. Several examples of stem cells and their regulation are summarized in Table 6.1. Because cells in different tissues have distinct transcriptional programs required for their specific functions, distinct regulatory mechanisms underlying self-renewal of different types of tissue stem cells may

Table 6.1 Signaling pathways regulating self-renewal, proliferation, and differentiation of stem cells

	Signaling pathways regulating self-renewal and proliferation	Signaling pathways regulating differentiation
Embryonic stem cell (mouse)	Wnt* (Young 2011), EGFR (Fricker-Gates et al. 2000), BMP* (Ying et al. 2003)	Notch (Lowell et al. 2006)
Embryonic stem cell (human)	EGFR (Fricker-Gates et al. 2000), TGF-β (James et al. 2005), bFGF (Wang et al. 2005)	Wnt* (Davidson et al. 2012; Kielman et al. 2002), BMP* (Pera et al. 2004)
Intestinal stem cell (*Drosophila*)	Wnt (Li and Xie 2005; Lin et al. 2008), EGFR (Jiang et al. 2011), JAK-STAT (Beebe et al. 2010), Hippo (Zhou et al. 2011), Integrin (O'Brien et al. 2011)	Notch (Ohlstein and Spradling 2007; Fre et al. 2005), JAK-STAT (Beebe et al. 2010), BMP (Auclair et al. 2007)
Intestinal stem cell (mammalian)	Wnt (Li and Xie 2005; Lin et al. 2008), Notch (mammalian)** (Pellegrinet et al. 2011)	Notch (Ohlstein and Spradling 2007; Fre et al. 2005), BMP (Auclair et al. 2007)
Hair follicle stem cell	Wnt (low) (Lowry et al. 2005), Hedgehog (Blanpain and Fuchs 2006)	Wnt (high) (Lowry et al. 2005), BMP, Notch (Blanpain and Fuchs 2006; Brack et al. 2008)
Hematopoietic stem cell	Wnt (Rattis et al. 2004), Notch (Butler et al. 2010)	Notch (Mercher et al. 2008)
Germline stem cell (*Drosophila* testis)	BMP (Kiger et al. 2001), JAK-STAT (Tulina and Matunis 2001), insulin (Ueishi et al. 2009)	
Cyst stem cell (*Drosophila* testis)	JAK-STAT (Stine and Matunis 2013), Hedgehog (Michel et al. 2012)	
Germline stem cell (*Drosophila* ovary)	BMP (Xie and Spradling 2000), Ecdysone (Ables and Drummond-Barbosa 2010), insulin (Ueishi et al. 2009)	
Follicle stem cell (*Drosophila* ovary)	Hedgehog (Zhang and Kalderon 2001), JAK-STAT (Vied et al. 2012), Wnt (Song and Xie 2003)	Notch (Adam and Montell 2004), Hippo (Chen et al. 2011)

(continued)

Table 6.1 (continued)

	Signaling pathways regulating self-renewal and proliferation	Signaling pathways regulating differentiation
Neural stem cell	Notch (Alvarez-Buylla and Lim 2004), EGFR (Aguirre et al. 2010), Hedgehog (Klein et al. 2001), JAK-STAT (Wang et al. 2011)	BMP (Shah et al. 1996)
Satellite muscle cell	Wnt7a (non-canonical) (Le Grand et al. 2009), Notch (Kuang et al. 2007)	Wnt (canonical) (Polesskaya et al. 2003)
Mammary stem cell	Wnt (Zeng and Nusse 2010), Hedgehog (Liu et al. 2006)	Notch (Dontu et al. 2004)
Airway stem cell	Wnt (Zhang et al. 2008), Notch (Paul et al. 2014)	Hippo (Zhao et al. 2014), Notch (Rock et al. 2011)

*Wnt and BMP have distinct functions in mouse and human ESCs; **Notch activation promotes ISC self-renewal in mammals, while inhibits ISC self-renewal in Drosophila

facilitate the execution of unique transcriptional programs required for cell-type-specific functions during cell lineage differentiation. Therefore, coupling stem cell identity with tissue identity could be an efficient and effective mechanism in controlling the self-renewal and differentiation of tissue stem cells.

Although signaling pathways involved in regulating various types of tissue stem cells have been elucidated and characterized, potential signaling cross talk or coordination for balanced self-renewal and differentiation is much less well understood. The downstream transcriptional factors that mediate signaling pathway-induced various stem cell behaviors are also in general less well understood. As signaling pathways have a central role in controlling stem cell properties and have been implicated in various diseases, further dissecting out their function and regulation in stem cells will not only contribute to our understanding of disease mechanisms, but also pave the way for regenerative medicine and drug discovery.

References

Aberle H, Bauer A, Stappert J, Kispert A, Kemler R. β-catenin is a target for the ubiquitin–proteasome pathway. EMBO J. 1997;16:3797–804.

Ables ET, Drummond-Barbosa D. The steroid hormone ecdysone functions with intrinsic chromatin remodeling factors to control female germline stem cells in *Drosophila*. Cell Stem Cell. 2010;7:581–92.

Adam JC, Montell DJ. A role for extra macrochaetae downstream of notch in follicle cell differentiation. Development. 2004;131:5971–80.

Aguirre A, Rubio ME, Gallo V. Notch and EGFR pathway interaction regulates neural stem cell number and self-renewal. Nature. 2010;467:323–7.

Akazawa C, Tsuzuki H, Nakamura Y, Sasaki Y, Ohsaki K, Nakamura S, Arakawa Y, Kohsaka S. The upregulated expression of sonic hedgehog in motor neurons after rat facial nerve axotomy. J Neurosci. 2004;24:7923–30.

Alarcón C, Zaromytidou A-I, Xi Q, Gao S, Yu J, Fujisawa S, Barlas A, Miller AN, Manova-Todorova K, Macias MJ. Nuclear CDKs drive smad transcriptional activation and turnover in BMP and TGF-β pathways. Cell. 2009;139:757–69.

Alexson TO, Hitoshi S, Coles BL, Bernstein A, van der Kooy D. Notch signaling is required to maintain all neural stem cell populations–irrespective of spatial or temporal niche. Dev Neurosci. 2006;28:34–48.

Alvarez-Buylla A, Lim DA. For the long run: maintaining germinal niches in the adult brain. Neuron. 2004;41:683–6.

Amcheslavsky A, Jiang J, Ip YT. Tissue damage-induced intestinal stem cell division in *Drosophila*. Cell Stem Cell. 2009; 4:49–61.

Amit S, Hatzubai A, Birman Y, Andersen JS, Ben-Shushan E, Mann M, Ben-Neriah Y, Alkalay I. Axin-mediated CKI phosphorylation of β-catenin at Ser 45: a molecular switch for the Wnt pathway. Genes Dev. 2002;16:1066–76.

Andersen P, Uosaki H, Shenje LT, Kwon C. Non-canonical notch signaling: emerging role and mechanism. Trends Cell Biol. 2012;22:257–65.

Andl T, Reddy ST, Gaddapara T, Millar SE. WNT signals are required for the initiation of hair follicle development. Dev Cell. 2002;2:643–53.

Aubert J, Dunstan H, Chambers I, Smith A. Functional gene screening in embryonic stem cells implicates Wnt antagonism in neural differentiation. Nat Biotechnol. 2002;20:1240–5.

Auclair BA, Benoit YD, Rivard N, Mishina Y, Perreault N. Bone morphogenetic protein signaling is essential for terminal differentiation of the intestinal secretory cell lineage. Gastroenterology. 2007;133:887–96.

Austin TW, Solar GP, Ziegler FC, Liem L, Matthews W. A role for the Wnt gene family in hematopoiesis: expansion of multilineage progenitor cells. Blood. 1997;89:3624–35.

Ayuso-Sacido A, Moliterno JA, Kratovac S, Kapoor GS, O'Rourke DM, Holland EC, Garcia-Verdugo JM, Roy NS, Boockvar JA. Activated EGFR signaling increases proliferation, survival, and migration and blocks neuronal differentiation in post-natal neural stem cells. J Neurooncol. 2010;97:323–37.

Barnabé-Heider F, Wasylnka JA, Fernandes KJ, Porsche C, Sendtner M, Kaplan DR, Miller FD. Evidence that embryonic neurons regulate the onset of cortical gliogenesis via cardiotrophin-1. Neuron. 2005;48:253–65.

Batlle E, Henderson JT, Beghtel H, van den Born MM, Sancho E, Huls G, Meeldijk J, Robertson J, van de Wetering M, Pawson T, Clevers H. Beta-catenin and TCF mediate cell positioning in the intestinal epithelium by controlling the expression of EphB/ephrinB. Cell. 2002;111:251–63.

Beebe K, Lee W-C, Micchelli CA. JAK/STAT signaling coordinates stem cell proliferation and multilineage differentiation in the *Drosophila* intestinal stem cell lineage. Dev Biol. 2010;338:28–37.

Beyer TA, Weiss A, Khomchuk Y, Huang K, Ogunjimi AA, Varelas X, Wrana JL. Switch enhancers interpret TGF-β and hippo signaling to control cell fate in human embryonic stem cells. Cell Rep. 2013;5:1611–24.

Bhardwaj G, Murdoch B, Wu D, Baker DP, Williams KP, Chadwick K, Ling LE, Karanu FN, Bhatia M. Sonic hedgehog induces the proliferation of primitive human hematopoietic cells via BMP regulation. Nat Immunol. 2001;2:172–80.

Bilić J, Huang Y-L, Davidson G, Zimmermann T, Cruciat C-M, Bienz M, Niehrs C. Wnt induces LRP6 signalosomes and promotes dishevelled-dependent LRP6 phosphorylation. Science. 2007;316:1619–22.

Birnie R, Bryce SD, Roome C, Dussupt V, Droop A, Lang SH, Berry PA, Hyde CF, Lewis JL, Stower MJ. Gene expression profiling of human prostate cancer stem cells reveals a pro-inflammatory phenotype and the importance of extracellular matrix interactions. Genome Biol. 2008;9:R83.

Biteau B, Jasper H. EGF signaling regulates the proliferation of intestinal stem cells in *Drosophila*. Development. 2011;138:1045–55.

Blanpain C, Fuchs E. Epidermal stem cells of the skin. Annu Rev Cell Dev Biol. 2006;22:339.
Blanpain C, Horsley V, Fuchs E. Epithelial stem cells: turning over new leaves. Cell. 2007;128:445–58.
Boockvar JA, Kapitonov D, Kapoor G, Schouten J, Counelis GJ, Bogler O, Snyder EY, McIntosh TK, O'Rourke DM. Constitutive EGFR signaling confers a motile phenotype to neural stem cells. Mol Cell Neurosci. 2003;24:1116–30.
Brack AS, Conboy IM, Conboy MJ, Shen J, Rando TA. A temporal switch from notch to Wnt signaling in muscle stem cells is necessary for normal adult myogenesis. Cell Stem Cell. 2008;2:50–9.
Bradley HL, Couldrey C, Bunting KD. Hematopoietic-repopulating defects from STAT5-deficient bone marrow are not fully accounted for by loss of thrombopoietin responsiveness. Blood. 2004;103:2965–72.
Brawley C, Matunis E. Regeneration of male germline stem cells by spermatogonial dedifferentiation in vivo. Science. 2004;304:1331–4.
Bray SJ. Notch signalling: a simple pathway becomes complex. Nat Rev Mol Cell Biol. 2006;7:678–89.
Briscoe J, Therond PP. The mechanisms of hedgehog signalling and its roles in development and disease. Nat Rev Mol Cell Biol. 2013;14:416–429.
Buchon N, Broderick NA, Chakrabarti S, Lemaitre B. Invasive and indigenous microbiota impact intestinal stem cell activity through multiple pathways in *Drosophila*. Genes Dev. 2009;23:2333–44.
Buchon N, Broderick NA, Kuraishi T, Lemaitre B. *Drosophila* EGFR pathway coordinates stem cell proliferation and gut remodeling following infection. BMC Biol. 2010;8:152.
Butler JM, Nolan DJ, Vertes EL, Varnum-Finney B, Kobayashi H, Hooper AT, Seandel M, Shido K, White IA, Kobayashi M. Endothelial cells are essential for the self-renewal and repopulation of notch-dependent hematopoietic stem cells. Cell Stem Cell. 2010;6:251–64.
Calvi L, Adams G, Weibrecht K, Weber J, Olson D, Knight M, Martin R, Schipani E, Divieti P, Bringhurst F. Osteoblastic cells regulate the haematopoietic stem cell niche. Nature. 2003;425:841–6.
Camargo FD, Gokhale S, Johnnidis JB, Fu D, Bell GW, Jaenisch R, Brummelkamp TR. YAP1 increases organ size and expands undifferentiated progenitor cells. Curr Biol. 2007;17:2054–60.
Cao X, Pfaff SL, Gage FH. YAP regulates neural progenitor cell number via the TEA domain transcription factor. Genes Dev. 2008;22:3320–34.
Cavallo RA, Cox RT, Moline MM, Roose J, Polevoy GA, Clevers H, Peifer M, Bejsovec A. *Drosophila* Tcf and groucho interact to repress wingless signalling activity. Nature. 1998;395:604–8.
Chambers I, Smith A. Self-renewal of teratocarcinoma and embryonic stem cells. Oncogene. 2004;23:7150–60.
Chen D, McKearin D. Dpp signaling silences bam transcription directly to establish asymmetric divisions of germline stem cells. Curr Biol. 2003;13:1786–91.
Chen C, Liu Y, Liu R, Ikenoue T, Guan K-L, Liu Y, Zheng P. TSC–mTOR maintains quiescence and function of hematopoietic stem cells by repressing mitochondrial biogenesis and reactive oxygen species. J Exp Med. 2008;205:2397–408.
Chen H, Wang C, Wang T, Liaw G, Hsu T, Lin T, Yu J. The Hippo pathway controls polar cell fate through Notch signaling during *Drosophila* oogenesis. Developmental biology. 2011;357:370.
Choi YS, Zhang Y, Xu M, Yang Y, Ito M, Peng T, Cui Z, Nagy A, Hadjantonakis AK, Lang RA, Cotsarelis G, Andl T, Morrisey EE, Millar SE. Distinct functions for Wnt/beta-catenin in hair follicle stem cell proliferation and survival and interfollicular epidermal homeostasis. Cell Stem Cell. 2013;13:720–33.
Cook AM, Li L, Ho Y, Lin A, Li L, Stein A, Forman S, Perrotti D, Jove R, Bhatia R. Role of altered growth factor receptor-mediated JAK2 signaling in growth and maintenance of human acute myeloid leukemia stem cells. Blood. 2014;123:2826–37.

Cooper MT, Bray SJ. R7 photoreceptor specification requires notch activity. Curr Biol. 2000;10:1507–10.

Cordenonsi M, Zanconato F, Azzolin L, Forcato M, Rosato A, Frasson C, Inui M, Montagner M, Parenti AR, Poletti A. The hippo transducer TAZ confers cancer stem cell-related traits on breast cancer cells. Cell. 2011;147:759–72.

Cordero JB, Stefanatos RK, Scopelliti A, Vidal M, Sansom OJ. Inducible progenitor-derived wingless regulates adult midgut regeneration in Drosophila. EMBO J. 2012;31:3901–17.

DasGupta R, Fuchs E. Multiple roles for activated LEF/TCF transcription complexes during hair follicle development and differentiation. Development. 1999;126:4557–68.

Davidson KC, Adams AM, Goodson JM, McDonald CE, Potter JC, Berndt JD, Biechele TL, Taylor RJ, Moon RT. Wnt/β-catenin signaling promotes differentiation, not self-renewal, of human embryonic stem cells and is repressed by Oct4. Proc Natl Acad Sci. 2012;109:4485–90.

Day TF, Guo X, Garrett-Beal L, Yang Y. Wnt/β-catenin signaling in mesenchymal progenitors controls osteoblast and chondrocyte differentiation during vertebrate skeletogenesis. Dev Cell. 2005;8:739–50.

de La Coste A, Six E, Fazilleau N, Mascarell L, Legrand N, Mailhé M-P, Cumano A, Laâbi Y, Freitas AA. In vivo and in absence of a thymus, the enforced expression of the notch ligands delta-1 or delta-4 promotes T cell development with specific unique effects. J Immunol. 2005;174:2730–7.

Dong J, Feldmann G, Huang J, Wu S, Zhang N, Comerford SA, Gayyed MF, Anders RA, Maitra A, Pan D. Elucidation of a universal size-control mechanism in *Drosophila* and Mammals. Cell. 2007;130:1120–33.

Dontu G, Jackson KW, McNicholas E, Kawamura MJ, Abdallah WM, Wicha MS. Role of Notch signaling in cell-fate determination of human mammary stem/progenitor cells. Breast Cancer Res. 2004;6:R605–15.

Dreesen O, Brivanlou AH. Signaling pathways in cancer and embryonic stem cells. Stem cell Rev. 2007;3:7–17.

Ernst M, Novak U, Nicholson SE, Layton JE, Dunn AR. The carboxyl-terminal domains of gp130-related cytokine receptors are necessary for suppressing embryonic stem cell differentiation involvement of stat3. J Biol Chem. 1999;274:9729–37.

Fang M, Li J, Blauwkamp T, Bhambhani C, Campbell N, Cadigan KM. C-terminal-binding protein directly activates and represses Wnt transcriptional targets in Drosophila. EMBO J. 2006;25:2735–45.

Fernandez L, Rodriguez S, Huang H, Chora A, Fernandes J, Mumaw C, Cruz E, Pollok K, Cristina F, Price JE, Ferkowicz MJ, Scadden DT, Clauss M, Cardoso AA, Carlesso N. Tumor necrosis factor-alpha and endothelial cells modulate notch signaling in the bone marrow microenvironment during inflammation. Exp Hematol. 2008;36:545–58.

Finch PW, He X, Kelley MJ, Uren A, Schaudies RP, Popescu NC, Rudikoff S, Aaronson SA, Varmus HE, Rubin JS. Purification and molecular cloning of a secreted, frizzled-related antagonist of Wnt action. Proc Natl Acad Sci USA. 1997;94:6770–5.

Firmbach-Kraft I, Byers M, Shows T, Dalla-Favera R, Krolewski J. tyk2, prototype of a novel class of non-receptor tyrosine kinase genes. Oncogene. 1990;5:1329–36.

Flores GV, Duan H, Yan H, Nagaraj R, Fu W, Zou Y, Noll M, Banerjee U. Combinatorial signaling in the specification of unique cell fates. Cell. 2000;103:75–85.

Fraguas S, Barberan S, Cebria F. EGFR signaling regulates cell proliferation, differentiation and morphogenesis during planarian regeneration and homeostasis. Dev Biol. 2011;354:87–101.

Fre S, Huyghe M, Mourikis P, Robine S, Louvard D, Artavanis-Tsakonas S. Notch signals control the fate of immature progenitor cells in the intestine. Nature. 2005;435:964–8.

Fre S, Hannezo E, Sale S, Huyghe M, Lafkas D, Kissel H, Louvi A, Greve J, Louvard D, Artavanis-Tsakonas S. Notch lineages and activity in intestinal stem cells determined by a new set of knock-in mice. PLoS ONE. 2011;6:e25785.

Freeman M. Reiterative use of the EGF receptor triggers differentiation of all cell types in the *Drosophila* eye. Cell. 1996;87:651–60.

Fricker-Gates RA, Winkler C, Kirik D, Rosenblad C, Carpenter MK, Bjorklund A. EGF infusion stimulates the proliferation and migration of embryonic progenitor cells transplanted in the adult rat striatum. Exp Neurol. 2000;165:237–47.

Gancz D, Gilboa L. Hormonal control of stem cell systems. Ann Rev Cell Dev Biol. 2013;29:137–62.

Gee ST, Milgram SL, Kramer KL, Conlon FL, Moody SA. Yes-associated protein 65 (YAP) expands neural progenitors and regulates Pax3 expression in the neural plate border zone. PLoS ONE. 2011;6:e20309.

Glinka A, Dolde C, Kirsch N, Huang YL, Kazanskaya O, Ingelfinger D, Boutros M, Cruciat CM, Niehrs C. LGR4 and LGR5 are R-spondin receptors mediating Wnt/β-catenin and Wnt/PCP signalling. EMBO Rep. 2011;12:1055–61.

Grewal SS. Insulin/TOR signaling in growth and homeostasis: a view from the fly world. Int J Biochem Cell Biol. 2009;41:1006–10.

Hadland BK, Huppert SS, Kanungo J, Xue Y, Jiang R, Gridley T, Conlon RA, Cheng AM, Kopan R, Longmore GD. A requirement for notch1 distinguishes 2 phases of definitive hematopoiesis during development. Blood. 2004;104:3097–105.

Hao J, Li T-G, Qi X, Zhao D-F, Zhao G-Q. WNT/β-catenin pathway up-regulates < i > Stat3 </i > and converges on LIF to prevent differentiation of mouse embryonic stem cells. Dev Biol. 2006;290:81–91.

Harrison C, Kiladjian J-J, Al-Ali HK, Gisslinger H, Waltzman R, Stalbovskaya V, McQuitty M, Hunter DS, Levy R, Knoops L. JAK inhibition with ruxolitinib versus best available therapy for myelofibrosis. N Engl J Med. 2012;366:787–98.

Hart S, Goh K, Novotny-Diermayr V, Hu C, Hentze H, Tan Y, Madan B, Amalini C, Loh Y, Ong L. SB1518, a novel macrocyclic pyrimidine-based JAK2 inhibitor for the treatment of myeloid and lymphoid malignancies. Leukemia. 2011;25:1751–9.

Harvey KF, Pfleger CM, Hariharan IK. The *Drosophila* mst ortholog, hippo restricts growth and cell proliferation and promotes apoptosis. Cell. 2003;114:457–67.

He F, Ge W, Martinowich K, Becker-Catania S, Coskun V, Zhu W, Wu H, Castro D, Guillemot F, Fan G. A positive autoregulatory loop of Jak-STAT signaling controls the onset of astrogliogenesis. Nat Neurosci. 2005;8:616–25.

Heallen T, Zhang M, Wang J, Bonilla-Claudio M, Klysik E, Johnson RL, Martin JF. Hippo pathway inhibits Wnt signaling to restrain cardiomyocyte proliferation and heart size. Science. 2011;332:458–61.

Hecht A, Vleminckx K, Stemmler MP, Van Roy F, Kemler R. The p300/CBP acetyltransferases function as transcriptional coactivators of β-catenin in vertebrates. EMBO J. 2000;19:1839–50.

Heitzler P. Chapter fourteen-biodiversity and noncanonical notch signaling. Curr Top Dev Biol. 2010;92:457–81.

Hitoshi S, Alexson T, Tropepe V, Donoviel D, Elia AJ, Nye JS, Conlon RA, Mak TW, Bernstein A, van der Kooy D. Notch pathway molecules are essential for the maintenance, but not the generation, of mammalian neural stem cells. Genes Dev. 2002;16:846–58.

Hsu H-J, Drummond-Barbosa D. Insulin levels control female germline stem cell maintenance via the niche in *Drosophila*. Proc Natl Acad Sci. 2009;106:1117–21.

Hsu H, Drummond-Barbosa D. Insulin signals control the competence of the *Drosophila* female germline stem cell niche to respond to notch ligands. Dev Biol. 2011;350:290.

Hsu H, LaFever L, Drummond-Barbosa D. Diet controls normal and tumorous germline stem cells via insulin-dependent and-independent mechanisms in *Drosophila*. Dev Biol. 2008;313:700–12.

Huang J, Wu S, Barrera J, Matthews K, Pan D. The hippo signaling pathway coordinately regulates cell proliferation and apoptosis by inactivating yorkie, the *Drosophila* homolog of YAP. Cell. 2005;122:421–34.

Humphrey RK, Beattie GM, Lopez AD, Bucay N, King CC, Firpo MT, Rose-John S, Hayek A. Maintenance of pluripotency in human embryonic stem cells is STAT3 independent. Stem Cells. 2004;22:522–30.

Imokawa Y, Yoshizato K. Expression of Sonic hedgehog gene in regenerating newt limb blastemas recapitulates that in developing limb buds. Proc Natl Acad Sci USA. 1997;94:9159–64.
James D, Levine AJ, Besser D, Hemmati-Brivanlou A. TGFbeta/activin/nodal signaling is necessary for the maintenance of pluripotency in human embryonic stem cells. Development. 2005;132:1273–82.
Jamieson CH, Ailles LE, Dylla SJ, Muijtjens M, Jones C, Zehnder JL, Gotlib J, Li K, Manz MG, Keating A. Granulocyte–macrophage progenitors as candidate leukemic stem cells in blast-crisis CML. N Engl J Med. 2004;351:657–67.
Jang J, Ku SY, Kim JE, Choi K, Kim YY, Kim HS, Oh SK, Lee EJ, Cho HJ, Song YH. Notch inhibition promotes human embryonic stem cell-derived cardiac mesoderm differentiation. Stem Cells. 2008;26:2782–90.
Jansson L, Larsson J. Normal hematopoietic stem cell function in mice with enforced expression of the hippo signaling effector YAP1. PLoS ONE. 2012;7:e32013.
Jeong H, Bae S, An SY, Byun MR, Hwang J-H, Yaffe MB, Hong J-H, Hwang ES. TAZ as a novel enhancer of MyoD-mediated myogenic differentiation. FASEB J. 2010;24:3310–20.
Jia J, Zhang W, Wang B, Trinko R, Jiang J. The *Drosophila* Ste20 family kinase dMST functions as a tumor suppressor by restricting cell proliferation and promoting apoptosis. Genes Dev. 2003;17:2514–9.
Jiang H, Patel PH, Kohlmaier A, Grenley MO, McEwen DG, Edgar BA. Cytokine/Jak/Stat signaling mediates regeneration and homeostasis in the *Drosophila* midgut. Cell. 2009;137:1343–1355.
Jiang H, Grenley MO, Bravo MJ, Blumhagen RZ, Edgar BA. EGFR/Ras/MAPK signaling mediates adult midgut epithelial homeostasis and regeneration in *Drosophila*. Cell Stem Cell. 2011;8:84–95.
Justice RW, Zilian O, Woods DF, Noll M, Bryant PJ. The *Drosophila* tumor suppressor gene warts encodes a homolog of human myotonic dystrophy kinase and is required for the control of cell shape and proliferation. Genes Dev. 1995;9:534–46.
Karanu FN, Murdoch B, Gallacher L, Wu DM, Koremoto M, Sakano S, Bhatia M. The notch ligand jagged-1 represents a novel growth factor of human hematopoietic stem cells. J Exp Med. 2000;192:1365–72.
Karanu FN, Murdoch B, Miyabayashi T, Ohno M, Koremoto M, Gallacher L, Wu D, Itoh A, Sakano S, Bhatia M. Human homologues of delta-1 and delta-4 function as mitogenic regulators of primitive human hematopoietic cells. Blood. 2001;97:1960–7.
Kato Y, Iwama A, Tadokoro Y, Shimoda K, Minoguchi M, Akira S, Tanaka M, Miyajima A, Kitamura T, Nakauchi H. Selective activation of STAT5 unveils its role in stem cell self-renewal in normal and leukemic hematopoiesis. J Exp Med. 2005;202:169–79.
Katoh Y, Katoh M. Hedgehog signaling pathway and gastrointestinal stem cell signaling network (review). Int J Mol Med. 2006;18:1019–23.
Kharas MG, Okabe R, Ganis JJ, Gozo M, Khandan T, Paktinat M, Gilliland DG, Gritsman K. Constitutively active AKT depletes hematopoietic stem cells and induces leukemia in mice. Blood. 2010;115:1406–15.
Kielman MF, Rindapää M, Gaspar C, van Poppel N, Breukel C, van Leeuwen S, Taketo MM, Roberts S, Smits R, Fodde R. Apc modulates embryonic stem-cell differentiation by controlling the dosage of β-catenin signaling. Nat Genet. 2002;32:594–605.
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;294:2542–5.
Klaus A, Birchmeier W. Wnt signalling and its impact on development and cancer. Nat Rev Cancer. 2008;8:387–98.
Klein RS, Rubin JB, Gibson HD, DeHaan EN, Alvarez-Hernandez X, Segal RA, Luster AD. SDF-1α induces chemotaxis and enhances Sonic hedgehog-induced proliferation of cerebellar granule cells. Development. 2001;128:1971–81.
Kobayashi H, Butler JM, O'Donnell R, Kobayashi M, Ding B-S, Bonner B, Chiu VK, Nolan DJ, Shido K, Benjamin L. Angiocrine factors from Akt-activated endothelial cells balance self-renewal and differentiation of haematopoietic stem cells. Nat Cell Biol. 2010;12:1046–56.

König A, Yatsenko AS, Weiss M, Shcherbata HR. Ecdysteroids affect *Drosophila* ovarian stem cell niche formation and early germline differentiation. EMBO J. 2011;30:1549–62.
Kopan R, Ilagan MXG. The canonical notch signaling pathway: unfolding the activation mechanism. Cell. 2009;137:216–33.
Korkaya H, Paulson A, Charafe-Jauffret E, Ginestier C, Brown M, Dutcher J, Clouthier SG, Wicha MS. Regulation of mammary stem/progenitor cells by PTEN/Akt/beta-catenin signaling. PLoS Biol. 2009;7:e1000121.
Krzemień J, Dubois L, Makki R, Meister M, Vincent A, Crozatier M. Control of blood cell homeostasis in *Drosophila* larvae by the posterior signalling centre. Nature. 2007;446:325–8.
Kuang S, Kuroda K, Le Grand F, Rudnicki MA. Asymmetric self-renewal and commitment of satellite stem cells in muscle. Cell. 2007;129:999–1010.
Kumano K, Chiba S, Kunisato A, Sata M, Saito T, Nakagami-Yamaguchi E, Yamaguchi T, Masuda S, Shimizu K, Takahashi T. Notch1 but not notch2 is essential for generating hematopoietic stem cells from endothelial cells. Immunity. 2003;18:699–711.
Laforest L, Brown CW, Poleo G, Geraudie J, Tada M, Ekker M, Akimenko MA. Involvement of the sonic hedgehog, patched 1 and bmp2 genes in patterning of the zebrafish dermal fin rays. Development. 1998;125:4175–84.
Lai K, Kaspar BK, Gage FH, Schaffer DV. Sonic hedgehog regulates adult neural progenitor proliferation in vitro and in vivo. Nat Neurosci. 2003;6:21–7.
Lai Z-C, Wei X, Shimizu T, Ramos E, Rohrbaugh M, Nikolaidis N, Ho L-L, Li Y. Control of cell proliferation and apoptosis by mob as tumor suppressor, mats. Cell. 2005;120:675–85.
Le Grand F, Jones AE, Seale V, Scimè A, Rudnicki MA. Wnt7a activates the planar cell polarity pathway to drive the symmetric expansion of satellite stem cells. Cell Stem Cell. 2009;4:535–47.
Leatherman JL, DiNardo S. Germline self-renewal requires cyst stem cells and stat regulates niche adhesion in *Drosophila* testes. Nat Cell Biol. 2010;12:806–11.
Leatherman JL, DiNardo S. Zfh-1 controls somatic stem cell self-renewal in the *Drosophila* testis and nonautonomously influences germline stem cell self-renewal. Cell stem cell. 2008;3:44–54.
Lee H-Y, Kléber M, Hari L, Brault V, Suter U, Taketo MM, Kemler R, Sommer L. Instructive role of Wnt/ß-catenin in sensory fate specification in neural crest stem cells. Science. 2004;303:1020–3.
Lee K-P, Lee J-H, Kim T-S, Kim T-H, Park H-D, Byun J-S, Kim M-C, Jeong W-I, Calvisi DF, Kim J-M. The hippo-salvador pathway restrains hepatic oval cell proliferation, liver size, and liver tumorigenesis. Proc Natl Acad Sci. 2010;107:8248–53.
Li L, Xie T. Stem cell niche: structure and function. Annu Rev Cell Dev Biol. 2005;21:605–31.
Lian I, Kim J, Okazawa H, Zhao J, Zhao B, Yu J, Chinnaiyan A, Israel MA, Goldstein LS, Abujarour R. The role of YAP transcription coactivator in regulating stem cell self-renewal and differentiation. Genes Dev. 2010;24:1106–18.
Liang K, Zhou G, Zhang Q, Li J, Zhang C. Expression of hippo pathway in colorectal cancer. Saudi J Gastroenterol. 2014;20:188.
Lillien L, Raphael H. BMP and FGF regulate the development of EGF-responsive neural progenitor cells. Development. 2000;127:4993–5005.
Lin H. The stem-cell niche theory: lessons from flies. Nat Rev Genet. 2002;3:931–40.
Lin G, Xu N, Xi R. Paracrine wingless signalling controls self-renewal of *Drosophila* intestinal stem cells. Nature. 2008;455:1119–23.
Lin G, Xu N, Xi R. Paracrine unpaired signaling through the JAK/STAT pathway controls self-renewal and lineage differentiation of *Drosophila* intestinal stem cells. J Mol Cell Biol. 2010;2:37–49.
Lindsley RC, Gill JG, Kyba M, Murphy TL, Murphy KM. Canonical Wnt signaling is required for development of embryonic stem cell-derived mesoderm. Development. 2006;133:3787–96.
Liu C, Li Y, Semenov M, Han C, Baeg G-H, Tan Y, Zhang Z, Lin X, He X. Control of β-catenin phosphorylation/degradation by a dual-kinase mechanism. Cell. 2002;108:837–47.

Liu S, Dontu G, Mantle ID, Patel S, Ahn N-S, Jackson KW, Suri P, Wicha MS. Hedgehog signaling and Bmi-1 regulate self-renewal of normal and malignant human mammary stem cells. Cancer Res. 2006;66:6063–71.

Liu J, Sato C, Cerletti M, Wagers A. Notch signaling in the regulation of stem cell self-renewal and differentiation. Curr Top Dev Biol. 2010a;92:367–409.

Liu W, Singh SR, Hou SX. JAK–STAT is restrained by notch to control cell proliferation of the *Drosophila* intestinal stem cells. J Cell Biochem. 2010b;109:992–9.

Lowell S, Benchoua A, Heavey B, Smith AG. Notch promotes neural lineage entry by pluripotent embryonic stem cells. PLoS Biol. 2006;4:e121.

Lowry WE, Blanpain C, Nowak JA, Guasch G, Lewis L, Fuchs E. Defining the impact of beta-catenin/Tcf transactivation on epithelial stem cells. Genes Dev. 2005;19:1596–611.

Machold R, Hayashi S, Rutlin M, Muzumdar MD, Nery S, Corbin JG, Gritli-Linde A, Dellovade T, Porter JA, Rubin LL, Dudek H, McMahon AP, Fishell G. Sonic hedgehog is required for progenitor cell maintenance in telencephalic stem cell niches. Neuron. 2003;39:937–50.

Mahoney W, Hong J, Yaffe M, Farrance I. The transcriptional co-activator TAZ interacts differentially with transcriptional enhancer factor-1 (TEF-1) family members. Biochem J. 2005;388:217–25.

Maillard I, Koch U, Dumortier A, Shestova O, Xu L, Sai H, Pross SE, Aster JC, Bhandoola A, Radtke F. Canonical notch signaling is dispensable for the maintenance of adult hematopoietic stem cells. Cell Stem Cell. 2008;2:356–66.

Makki R, Meister M, Pennetier D, Ubeda J-M, Braun A, Daburon V, Krzemień J, Bourbon H-M, Zhou R, Vincent A. A short receptor downregulates JAK/STAT signalling to control the *Drosophila* cellular immune response. PLoS Biol. 2010;8:e1000441.

Mancini SJ, Mantei N, Dumortier A, Suter U, MacDonald HR, Radtke F. Jagged1-dependent notch signaling is dispensable for hematopoietic stem cell self-renewal and differentiation. Blood. 2005;105:2340–2.

Mascarenhas JO, Cross NC, Mesa RA. The future of JAK inhibition in myelofibrosis and beyond. Blood Rev. 2014;28:189.

Mercher T, Cornejo MG, Sears C, Kindler T, Moore SA, Maillard I, Pear WS, Aster JC, Gilliland DG. Notch signaling specifies megakaryocyte development from hematopoietic stem cells. Cell Stem Cell. 2008;3:314–26.

Micchelli CA, Perrimon N. Evidence that stem cells reside in the adult *Drosophila* midgut epithelium. Nature. 2005;439:475–9.

Michel M, Kupinski AP, Raabe I, Bökel C. Hh signalling is essential for somatic stem cell maintenance in the *Drosophila* testis niche. Development. 2012;139:2663–9.

Milano J, McKay J, Dagenais C, Foster-Brown L, Pognan F, Gadient R, Jacobs RT, Zacco A, Greenberg B, Ciaccio PJ. Modulation of notch processing by γ-secretase inhibitors causes intestinal goblet cell metaplasia and induction of genes known to specify gut secretory lineage differentiation. Toxicol Sci. 2004;82:341–58.

Morgan TH. The theory of the gene. Am Nat. 1917;51:513–44.

Morris LX, Spradling AC. Steroid signaling within *Drosophila* ovarian epithelial cells sex-specifically modulates early germ cell development and meiotic entry. PLoS ONE. 2012;7:e46109.

Morrison SJ, Spradling AC. Stem cells and niches: mechanisms that promote stem cell maintenance throughout life. Cell. 2008;132:598–611.

Mosimann C, Hausmann G, Basler K. Parafibromin/Hyrax activates Wnt/Wg target gene transcription by direct association with β-catenin/Armadillo. Cell. 2006;125:327–41.

Muller M, Briscoe J, Laxton C, Guschin D, Ziemiecki A, Silvennoinen O, Harpur AG, Barbieri G, Witthuhn BA, Schindler C, et al. The protein tyrosine kinase JAK1 complements defects in interferon-alpha/beta and -gamma signal transduction. Nature. 1993;366:129–35.

Murry CE, Keller G. Differentiation of embryonic stem cells to clinically relevant populations: lessons from embryonic development. Cell. 2008;132:661–80.

Nejigane S, Takahashi S, Haramoto Y, Michiue T, Asashima M. Hippo signaling components, Mst1 and Mst2, act as a switch between self-renewal and differentiation in xenopus hematopoietic and endothelial progenitors. Int J Dev Biol. 2013;57:407–14.

Nemir M, Croquelois A, Pedrazzini T, Radtke F. Induction of cardiogenesis in embryonic stem cells via downregulation of notch1 signaling. Circ Res. 2006;98:1471–8.

Ngo KT, Wang J, Junker M, Kriz S, Vo G, Asem B, Olson JM, Banerjee U, Hartenstein V. Concomitant requirement for Notch and Jak/Stat signaling during neuro-epithelial differentiation in the *Drosophila* optic lobe. Dev Biol. 2010;346:284–295.

Nishioka N, Inoue K-I, Adachi K, Kiyonari H, Ota M, Ralston A, Yabuta N, Hirahara S, Stephenson RO, Ogonuki N. The hippo signaling pathway components lats and yap pattern tead4 activity to distinguish mouse trophectoderm from inner cell mass. Dev Cell. 2009;16:398–410.

Noggle SA, Weiler D, Condie BG. Notch signaling is inactive but inducible in human embryonic stem cells. Stem Cells. 2006;24:1646–53.

Nusse R, Varmus HE. Many tumors induced by the mouse mammary tumor virus contain a provirus integrated in the same region of the host genome. Cell. 1982;31:99–109.

Nusse R, Brown A, Papkoff J, Scambler P, Shackleford G, McMahon A, Moon R, Varmus H. A new nomenclature for int-1 and related genes: the Wnt gene family. Cell. 1991;64:231.

Oatley JM, Kaucher AV, Avarbock MR, Brinster RL. Regulation of mouse spermatogonial stem cell differentiation by STAT3 signaling. Biol Reprod. 2010;83:427–33.

O'Brien LE, Soliman SS, Li X, Bilder D. Altered modes of stem cell division drive adaptive intestinal growth. Cell. 2011;147:603–14.

Oh H, Irvine KD. In vivo regulation of yorkie phosphorylation and localization. Development. 2008;135:1081–8.

Ohlstein B, Spradling A. The adult *Drosophila* posterior midgut is maintained by pluripotent stem cells. Nature. 2005;439:470–4.

Ohlstein B, Spradling A. Multipotent *Drosophila* intestinal stem cells specify daughter cell fates by differential notch signaling. Science. 2007;315:988–92.

Onishi K, Tonge PD, Nagy A, Zandstra PW. Microenvironment-mediated reversion of epiblast stem cells by reactivation of repressed JAK–STAT signaling. Integr Biol. 2012;4:1367–76.

Pantalacci S, Tapon N, Léopold P. The salvador partner hippo promotes apoptosis and cell-cycle exit in *Drosophila*. Nat Cell Biol. 2003;5:921–7.

Pardanani A, Laborde R, Lasho T, Finke C, Begna K, Al-Kali A, Hogan W, Litzow M, Leontovich A, Kowalski M. Safety and efficacy of CYT387, a JAK1 and JAK2 inhibitor, in myelofibrosis. Leukemia. 2013;27:1322–7.

Paul MK, Bisht B, Darmawan DO, Chiou R, Ha VL, Wallace WD, Chon AT, Hegab AE, Grogan T, Elashoff DA, Alva-Ornelas JA, Gomperts BN. Dynamic changes in intracellular ROS levels regulate airway basal stem cell homeostasis through Nrf2-dependent notch signaling. Cell Stem Cell. 2014;15:199–214.

Pellegrinet L, Rodilla V, Liu Z, Chen S, Koch U, Espinosa L, Kaestner KH, Kopan R, Lewis J, Radtke F. Dll1-and dll4-mediated notch signaling are required for homeostasis of intestinal stem cells. Gastroenterology. 2011;140:1230–1240. e1237.

Pera MF, Trounson AO. Human embryonic stem cells: prospects for development. Development. 2004;131:5515–25.

Pera MF, Andrade J, Houssami S, Reubinoff B, Trounson A, Stanley EG, Ward-van Oostwaard D, Mummery C. Regulation of human embryonic stem cell differentiation by BMP-2 and its antagonist noggin. J Cell Sci. 2004;117:1269–80.

Perdigoto CN, Bardin AJ. Sending the right signal: Notch and stem cells. Biochim Biophys Acta (BBA)-Gen Subj. 2013;1830:2307–2322.

Perra A, Kowalik MA, Ghiso E, Ledda-Columbano GM, Di Tommaso L, Angioni MM, Raschioni C, Testore E, Roncalli M, Giordano S. YAP activation is an early event and a potential therapeutic target in liver cancer development. J Hepatol. 2014;61:1088.

Peters JM, McKay RM, McKay JP, Graff JM. Casein kinase I transduces Wnt signals. Nature. 1999;401:345–50.

Phelps RA, Chidester S, Dehghanizadeh S, Phelps J, Sandoval IT, Rai K, Broadbent T, Sarkar S, Burt RW, Jones DA. A two-step model for colon adenoma initiation and progression caused by APC loss. Cell. 2009;137:623–34.

Pinto D, Gregorieff A, Begthel H, Clevers H. Canonical Wnt signals are essential for homeostasis of the intestinal epithelium. Genes Dev. 2003;17:1709–13.

Polesskaya A, Seale P, Rudnicki MA. Wnt signaling induces the myogenic specification of resident CD45 + adult stem cells during muscle regeneration. Cell. 2003;113:841–52.

Radtke F, Wilson A, Stark G, Bauer M, van Meerwijk J, MacDonald HR, Aguet M. Deficient T cell fate specification in mice with an induced inactivation of notch1. Immunity. 1999;10:547–58.

Radtke F, Wilson A, Mancini SJ, MacDonald HR. Notch regulation of lymphocyte development and function. Nat Immunol. 2004;5:247–53.

Rakesh K, Agrawal DK. Controlling cytokine signaling by constitutive inhibitors. Biochem Pharmacol. 2005;70:649–57.

Ramos A, Camargo FD. The hippo signaling pathway and stem cell biology. Trends Cell Biol. 2012;22:339–46.

Rattis FM, Voermans C, Reya T. Wnt signaling in the stem cell niche. Curr Opin Hematol. 2004;11:88–94.

Ren F, Zhang L, Jiang J. Hippo signaling regulates yorkie nuclear localization and activity through 14-3-3 dependent and independent mechanisms. Dev Biol. 2010;337:303–12.

Riccio O, van Gijn ME, Bezdek AC, Pellegrinet L, van Es JH, Zimber-Strobl U, Strobl LJ, Honjo T, Clevers H, Radtke F. Loss of intestinal crypt progenitor cells owing to inactivation of both Notch1 and Notch2 is accompanied by derepression of CDK inhibitors p27Kip1 and p57Kip2. EMBO Rep. 2008;9:377–83.

Rijsewijk F, Schuermann M, Wagenaar E, Parren P, Weigel D, Nusse R. The *Drosophila* homolog of the mouse mammary oncogene int-1 is identical to the segment polarity gene wingless. Cell. 1987;50:649–57.

Robert-Moreno À, Guiu J, Ruiz-Herguido C, López ME, Inglés-Esteve J, Riera L, Tipping A, Enver T, Dzierzak E, Gridley T. Impaired embryonic haematopoiesis yet normal arterial development in the absence of the Notch ligand Jagged1. EMBO J. 2008;27:1886–95.

Rock JR, Gao X, Xue Y, Randell SH, Kong Y-Y, Hogan BL. Notch-dependent differentiation of adult airway basal stem cells. Cell Stem Cell. 2011;8:639–48.

Roy S, Gardiner DM. Cyclopamine induces digit loss in regenerating axolotl limbs. J Exp Zool. 2002;293:186–90.

Ryan MA, Nattamai KJ, Xing E, Schleimer D, Daria D, Sengupta A, Kohler A, Liu W, Gunzer M, Jansen M, Ratner N, Le Cras TD, Waterstrat A, Van Zant G, Cancelas JA, Zheng Y, Geiger H. Pharmacological inhibition of EGFR signaling enhances G-CSF-induced hematopoietic stem cell mobilization. Nat Med. 2010;16:1141–6.

Sato N, Meijer L, Skaltsounis L, Greengard P, Brivanlou AH. Maintenance of pluripotency in human and mouse embryonic stem cells through activation of Wnt signaling by a pharmacological GSK-3-specific inhibitor. Nat Med. 2003;10:55–63.

Schlegelmilch K, Mohseni M, Kirak O, Pruszak J, Rodriguez JR, Zhou D, Kreger BT, Vasioukhin V, Avruch J, Brummelkamp TR. Yap1 acts downstream of α-catenin to control epidermal proliferation. Cell. 2011;144:782–95.

Schofield R. The relationship between the spleen colony-forming cell and the haemopoietic stem cell. Blood Cells. 1977;4:7–25.

Schroeder T, Fraser ST, Ogawa M, Nishikawa S, Oka C, Bornkamm GW, Nishikawa S-I, Honjo T, Just U. Recombination signal sequence-binding protein Jκ alters mesodermal cell fate decisions by suppressing cardiomyogenesis. Proc Natl Acad Sci. 2003;100:4018–23.

Seifert JR, Mlodzik M. Frizzled/PCP signalling: a conserved mechanism regulating cell polarity and directed motility. Nat Rev Genet. 2007;8:126–38.

Shah NM, Groves AK, Anderson DJ. Alternative neural crest cell fates are instructively promoted by TGFbeta superfamily members. Cell. 1996;85:331–43.

Sherry MM, Reeves A, Wu JK, Cochran BH. STAT3 is required for proliferation and maintenance of multipotency in glioblastoma stem cells. Stem Cells. 2009;27:2383–92.
Shuai K, Ziemiecki A, Wilks AF, Harpur AG, Sadowski HB, Gilman MZ, Darnell JE. Polypeptide signalling to the nucleus through tyrosine phosphorylation of jak and stat proteins. Nature. 1993;366:580.
Shuai K, Stark GR, Kerr IM, Darnell J. A single phosphotyrosine residue of stat91 required for gene activation by interferon-gamma. Science. 1993;261:1744–6.
Snow JW, Abraham N, Ma MC, Abbey NW, Herndier B, Goldsmith MA. STAT5 promotes multilineage hematolymphoid development in vivo through effects on early hematopoietic progenitor cells. Blood. 2002;99:95–101.
Song X, Xie T. Wingless signaling regulates the maintenance of ovarian somatic stem cells in *Drosophila*. Development. 2003;130:3259–68.
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;131:1353–64.
Song H, Mak KK, Topol L, Yun K, Hu J, Garrett L, Chen Y, Park O, Chang J, Simpson RM. Mammalian Mst1 and Mst2 kinases play essential roles in organ size control and tumor suppression. Proc Natl Acad Sci. 2010;107:1431–6.
Song S, Ajani JA, Honjo S, Maru DM, Chen Q, Scott AW, Heallen TR, Xiao L, Hofstetter WL, Weston B. Hippo coactivator YAP1 upregulates SOX9 and endows stem-like properties to esophageal cancer cells. Cancer Res. 2014;13:canres. 3569.2013.
Staley BK, Irvine KD. Hippo signaling in *Drosophila*: recent advances and insights. Dev Dyn. 2012;241:3–15.
Stanger BZ, Tanaka AJ, Melton DA. Organ size is limited by the number of embryonic progenitor cells in the pancreas but not the liver. Nature. 2007;445:886–91.
Steinhardt AA, Gayyed MF, Klein AP, Dong J, Maitra A, Pan D, Montgomery EA, Anders RA. Expression of yes-associated protein in common solid tumors. Hum Pathol. 2008;39:1582–9.
Stier S, Cheng T, Dombkowski D, Carlesso N, Scadden DT. Notch1 activation increases hematopoietic stem cell self-renewal in vivo and favors lymphoid over myeloid lineage outcome. Blood. 2002;99:2369–78.
Stine RR, Matunis EL. JAK-STAT signaling in stem cells. In: Transcriptional and translational regulation of stem cells. The Netherlands: Springer; 2013. pp. 247–267.
Stofanko M, Kwon SY, Badenhorst P. Lineage tracing of lamellocytes demonstrates *Drosophila* macrophage plasticity. PLoS ONE. 2010;5:e14051.
Tamm C, Böwer N, Annerén C. Regulation of mouse embryonic stem cell self-renewal by a Yes–YAP–TEAD2 signaling pathway downstream of LIF. J Cell Sci. 2011;124:1136–44.
Tang Y, Tian XC. JAK-STAT3 and somatic cell reprogramming. JAK-STAT. 2013;2:e24935–e24935.
Tang Y, Luo Y, Jiang Z, Ma Y, Lin CJ, Kim C, Carter MG, Amano T, Park J, Kish S. Jak/Stat3 signaling promotes somatic cell reprogramming by epigenetic regulation. Stem Cells. 2012;30:2645–56.
Tao W, Zhang S, Turenchalk GS, Stewart RA, St MA, Chen W, Xu T. Human homologue of the *Drosophila* melanogaster lats tumour suppressor modulates CDC2 activity. Nat Genet. 1999;21:177–81.
Tapon N, Harvey KF, Bell DW, Wahrer DC, Schiripo TA, Haber DA, Hariharan IK. Salvador promotes both cell cycle exit and apoptosis in *Drosophila* and is mutated in human cancer cell lines. Cell. 2002;110:467–478.
Toledo EM, Colombres M, Inestrosa NC. Wnt signaling in neuroprotection and stem cell differentiation. Prog Neurobiol. 2008;86:281–96.
Trompouki E, Bowman TV, Lawton LN, Fan ZP, Wu D-C, DiBiase A, Martin CS, Cech JN, Sessa AK, Leblanc JL. Lineage regulators direct BMP and Wnt pathways to cell-specific programs during differentiation and regeneration. Cell. 2011;147:577–89.
Tropepe V, Hitoshi S, Sirard C, Mak TW, Rossant J, van der Kooy D. Direct neural fate specification from embryonic stem cells: a primitive mammalian neural stem cell stage acquired through a default mechanism. Neuron. 2001;30:65–78.

Tsonis PA, Vergara MN, Spence JR, Madhavan M, Kramer EL, Call MK, Santiago WG, Vallance JE, Robbins DJ, Del Rio-Tsonis K. A novel role of the hedgehog pathway in lens regeneration. Dev Biol. 2004;267:450–61.

Tulina N, Matunis E. Control of stem cell self-renewal in *Drosophila* spermatogenesis by JAK-STAT signaling. Science. 2001;294:2546–9.

Udan RS, Kango-Singh M, Nolo R, Tao C, Halder G. Hippo promotes proliferation arrest and apoptosis in the salvador/warts pathway. Nat Cell Biol. 2003;5:914–20.

Ueishi S, Shimizu H, Inoue YH. Male germline stem cell division and spermatocyte growth require insulin signaling in *Drosophila*. Cell Struct Funct. 2009;34:61–9.

Ueo T, Imayoshi I, Kobayashi T, Ohtsuka T, Seno H, Nakase H, Chiba T, Kageyama R. The role of Hes genes in intestinal development, homeostasis and tumor formation. Development. 2012;139:1071–82.

Ungureanu D, Vanhatupa S, Kotaja N, Yang J, Aittomäki S, Jänne OA, Palvimo JJ, Silvennoinen O. PIAS proteins promote SUMO-1 conjugation to STAT1. Blood. 2003;102:3311–3.

Ungureanu D, Vanhatupa S, Grönholm J, Palvimo JJ, Silvennoinen O. SUMO-1 conjugation selectively modulates STAT1-mediated gene responses. Blood. 2005;106:224–6.

van Es JH, Jay P, Gregorieff A, van Gijn ME, Jonkheer S, Hatzis P, Thiele A, van den Born M, Begthel H, Brabletz T. Wnt signalling induces maturation of paneth cells in intestinal crypts. Nat Cell Biol. 2005;7:381–6.

Varelas X, Sakuma R, Samavarchi-Tehrani P, Peerani R, Rao BM, Dembowy J, Yaffe MB, Zandstra PW, Wrana JL. TAZ controls Smad nucleocytoplasmic shuttling and regulates human embryonic stem-cell self-renewal. Nat Cell Biol. 2008;10:837–48.

Varnum-Finney B, Brashem-Stein C, Bernstein ID. Combined effects of notch signaling and cytokines induce a multiple log increase in precursors with lymphoid and myeloid reconstituting ability. Blood. 2003;101:1784–9.

Vassilev A, Kaneko KJ, Shu H, Zhao Y, DePamphilis ML. TEAD/TEF transcription factors utilize the activation domain of YAP65, a Src/Yes-associated protein localized in the cytoplasm. Genes Dev. 2001;15:1229–41.

Veeman MT, Axelrod JD, Moon RT. A second canon: functions and mechanisms of β-catenin-independent Wnt signaling. Dev Cell. 2003;5:367–77.

Verstovsek S, Mesa RA, Gotlib J, Levy RS, Gupta V, DiPersio JF, Catalano JV, Deininger M, Miller C, Silver RT. A double-blind, placebo-controlled trial of ruxolitinib for myelofibrosis. N Engl J Med. 2012;366:799–807.

Vied C, Reilein A, Field NS, Kalderon D. Regulation of stem cells by intersecting gradients of long-range niche signals. Dev Cell. 2012;23:836–48.

von Gise A, Lin Z, Schlegelmilch K, Honor LB, Pan GM, Buck JN, Ma Q, Ishiwata T, Zhou B, Camargo FD. YAP1, the nuclear target of Hippo signaling, stimulates heart growth through cardiomyocyte proliferation but not hypertrophy. Proc Natl Acad Sci. 2012;109:2394–9.

Voog J, Jones DL. Stem cells and the niche: a dynamic duo. Cell Stem Cell. 2010;6:103–15.

Wang W, Li Y, Zhou L, Yue H, Luo H. Role of JAK/STAT signaling in neuroepithelial stem cell maintenance and proliferation in the *Drosophila* optic lobe. Biochem Biophys Res Commun.2011;410:714–720.

Wang G, Zhang H, Zhao Y, Li J, Cai J, Wang P, Meng S, Feng J, Miao C, Ding M, Li D, Deng H. Noggin and bFGF cooperate to maintain the pluripotency of human embryonic stem cells in the absence of feeder layers. Biochem Biophys Res Commun. 2005;330:934–42.

Wang Z, Li G, Tse W, Bunting KD. Conditional deletion of STAT5 in adult mouse hematopoietic stem cells causes loss of quiescence and permits efficient nonablative stem cell replacement. Blood. 2009;113:4856–65.

Watling D, Guschin D, Müller M, Silvennoinen O, Witthuhn BA, Quelle FW, Rogers NC, Schindler C, Stark GR, Ihle JN. Complementation by the protein tyrosine kinase JAK2 of a mutant cell line defective in the interferon-& gamma; signal transduction pathway. Nature. 1993;366:166.

Wilks A, Harpur A, Kurban R, Ralph S, Zürcher G, Ziemiecki A. Two novel protein-tyrosine kinases, each with a second phosphotransferase-related catalytic domain, define a new class of protein kinase. Mol Cell Biol. 1991;11:2057–65.

Wu S, Huang J, Dong J, Pan D. hippo encodes a ste-20 family protein kinase that restricts cell proliferation and promotes apoptosis in conjunction with salvador and warts. Cell. 2003;114:445–56.

Wu H, Wu Y, Ai Z, Yang L, Gao Y, Du J, Guo Z, Zhang Y. Vitamin C enhances nanog expression via activation of the JAK/STAT signaling pathway. Stem Cells. 2014;32:166–76.

Xie T, Spradling AC. A niche maintaining germ line stem cells in the *Drosophila* ovary. Science. 2000;290:328–30.

Xin M, Kim Y, Sutherland LB, Qi X, McAnally J, Schwartz RJ, Richardson JA, Bassel-Duby R, Olson EN. Regulation of insulin-like growth factor signaling by yap governs cardiomyocyte proliferation and embryonic heart size. Sci Signal. 2011; 4:ra70.

Xu T, Wang W, Zhang S, Stewart RA, Yu W. Identifying tumor suppressors in genetic mosaics: the *Drosophila* lats gene encodes a putative protein kinase. Development. 1995;121:1053–63.

Xu RH, Peck RM, Li DS, Feng X, Ludwig T, Thomson JA. Basic FGF and suppression of BMP signaling sustain undifferentiated proliferation of human ES cells. Nat Methods. 2005;2:185–90.

Xu N, Wang SQ, Tan D, Gao Y, Lin G, Xi R. EGFR, wingless and JAK/STAT signaling cooperatively maintain *Drosophila* intestinal stem cells. Dev Biol. 2011;354:31–43.

Yasugi T, Umetsu D, Murakami S, Sato M, Tabata T. *Drosophila* optic lobe neuroblasts triggered by a wave of proneural gene expression that is negatively regulated by JAK/STAT. Development. 2008;135:1471–80.

Yilmaz ÖH, Katajisto P, Lamming DW, Gültekin Y, Bauer-Rowe KE, Sengupta S, Birsoy K, Dursun A, Yilmaz VO, Selig M. mTORC1 in the Paneth cell niche couples intestinal stem-cell function to calorie intake. Nature. 2012;486:490.

Yimlamai D, Christodoulou C, Galli GG, Yanger K, Pepe-Mooney B, Gurung B, Shrestha K, Cahan P, Stanger BZ, Camargo FD. Hippo pathway activity influences liver cell fate. Cell. 2014;157:1324–38.

Ying QL, Smith AG. Defined conditions for neural commitment and differentiation. Methods Enzymol. 2003;365:327–41.

Ying QL, Nichols J, Chambers I, Smith A. BMP induction of Id proteins suppresses differentiation and sustains embryonic stem cell self-renewal in collaboration with STAT3. Cell. 2003a;115:281–92.

Ying QL, Stavridis M, Griffiths D, Li M, Smith A. Conversion of embryonic stem cells into neuroectodermal precursors in adherent monoculture. Nat Biotechnol. 2003b;21:183–6.

Young RA. Control of the embryonic stem cell state. Cell. 2011;144:940–54.

Yue G, Sun X, Gimenez-Capitan A, Shen J, Yu L, Teixido C, Guan W, Rosell R, Liu B, Wei J. TAZ is highly expressed in gastric signet ring cell carcinoma. BioMed Res Int. 2014; 2014:6.

Zeng YA, Nusse R. Wnt proteins are self-renewal factors for mammary stem cells and promote their long-term expansion in culture. Cell Stem Cell. 2010;6:568–77.

Zhang Y, Kalderon D. Hedgehog acts as a somatic stem cell factor in the *Drosophila* ovary. Nature. 2001;410:599–604.

Zhang Y, Goss AM, Cohen ED, Kadzik R, Lepore JJ, Muthukumaraswamy K, Yang J, DeMayo FJ, Whitsett JA, Parmacek MS. A Gata6-Wnt pathway required for epithelial stem cell development and airway regeneration. Nat Genet. 2008;40:862–70.

Zhao R, Fallon TR, Saladi SV, Pardo-Saganta A, Villoria J, Mou H, Vinarsky V, Gonzalez-Celeiro M, Nunna N, Hariri LP, Camargo F, Ellisen LW, Rajagopal J. Yap tunes airway epithelial size and architecture by regulating the identity, maintenance, and self-renewal of stem cells. Dev Cell. 2014;30:151–65.

Zhou J, Wulfkuhle J, Zhang H, Gu P, Yang Y, Deng J, Margolick JB, Liotta LA, Petricoin E, Zhang Y. Activation of the PTEN/mTOR/STAT3 pathway in breast cancer stem-like cells is required for viability and maintenance. Proc Natl Acad Sci. 2007;104:16158–63.

Zhou D, Zhang Y, Wu H, Barry E, Yin Y, Lawrence E, Dawson D, Willis JE, Markowitz SD, Camargo FD. Mst1 and Mst2 protein kinases restrain intestinal stem cell proliferation and colonic tumorigenesis by inhibition of Yes-associated protein (Yap) overabundance. Proc Natl Acad Sci. 2011;108:E1312–20.

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