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Review

BMP signaling and stem cell regulation

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Abstract

Stem cells play an essential role in cellular specialization and pattern formation during embryogenesis and in tissue regeneration in adults. This is mainly due to a stem cell's ability to replenish itself (self-renewal) and, at the same time, produce differentiated progeny. Realization of these special stem cell features has changed the prospective of the field. However, regulation of stem cell self-renewal and maintenance of its potentiality require a complicated regulatory network of both extracellular cues and intrinsic programs. Understanding how signaling regulates stem cell behavior will shed light on the molecular mechanisms underlying stem cell self-renewal. In this review, we focus on comparing the progress of recent research regarding the roles of the BMP signaling pathway in different stem cell systems, including embryonic stem cells, germline stem cells, hematopoietic stem cells, and intestinal stem cells. We hope this comparison, together with a brief look at other signaling pathways, will bring a more balanced view of BMP signaling in regulation of stem cell properties, and further point to a general principle that self-renewal of stem cells may require a combination of maintenance of proliferation potential, inhibition of apoptosis, and blocking of differentiation.

Keywords: BMP; Stem cell; Embryonic stem cell; Germline stem cell; Hematopoietic stem cell; Intestinal stem cell

Introduction

Stem cells are the key subset of cells functioning as ancestor cells to produce a variety of types of functionally specialized mature cells in a given tissue, while at the same time undergoing self-renewal, a process of reproducing themselves without losing their developmental potentiality. This self-renewal process is controlled by intrinsic genetic pathways that are subject to regulation by extrinsic signals from the microenvironment (called niche) in which stem cells reside. Stem cells play essential roles ranging from embryonic development and organogenesis (embryonic/fetal stem cells) to tissue regeneration (adult stem cells) (Lin, 2003; Spradling et al., 2001; Watt and Hogan, 2000; Weissman, 2000). To maintain homeostasis, a precise balance between self-renewal and differentiation of stem cells is essential. Loss of this balance tends to lead to uncontrolled cell growth or pre-maturation and thus results in tumors, cancers, or tissue defects. Therefore, understanding the complex signal regulation of stem cell development is crucial for future therapeutic applications. In this review, we will focus on progress that has been made in research studying the *b*one *m*orphogenesis *p*rotein (BMP) signaling pathway in regulation of stem cell properties.

BMPs belong to the *t*ransformation *g*rowth *f*actor beta (TGFβ) superfamily. They are involved in regulation of cell proliferation, differentiation, and apoptosis and therefore play essential roles during embryonic development and pattern formation (Massague, 1998). To maintain homeostasis in adults, the BMP signal also participates in tissue remodeling and regeneration, in which regulation of stem cell behavior is prominent.

There are more than 20 BMPs. Some BMPs have a distinct function while others have overlapping functions, depending on the specificity of their interaction with different types of receptors and the tissues in which they

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are differentially expressed (Mishina, 2003). Accumulated evidence indicates that BMPs play an important role in regulation of stem cell properties; however, their functions are different in the different stem cell compartments. For instance, in *Drosophila* *g*ermline *s*tem *c*ells (GSCs), Dpp (homolog of BMP2/4) is essential for the maintenance of stem cells (Xie and Spradling, 1998); in *e*mbryonic *s*tem *c*ells (ESCs), BMP signaling appears to be required for ESC self-renewal but this is owing to its ability to block neural differentiation (Ying et al., 2003a) in addition to its ability to promote non-neural (mesoderm and trophoblast) differentiation (Xu et al., 2002; Ying et al., 2003a); in mesenchymal stem cells, the BMP signal induces osteoblastic differentiation through Bmpr1b but inhibits osteoblastic differentiation through Bmpr1a (Chen et al., 1998); in intestinal stem cells (ISCs), BMP signaling inhibits stem cell activation and expansion (He et al., 2004); and in hematopoietic stem cells (HSCs), BMP signaling through Bmpr1a restricts stem cell number by controlling the niche size (Zhang et al., 2003). A critical and comparative review of the roles of BMPs in different settings and in different stem cell compartments is necessary for a balanced view towards BMP function in the regulation of stem cell properties, and thus will provide important insight into understanding the complex signaling regulation of stem cell self-renewal and fate determination.

Stem cell self-renewal—an event independent of other cellular events or the result of a combination of other cellular events?

The molecular mechanisms that control stem cell self-renewal remain largely unknown, albeit a large body of literature has been published with regard to stem cell self-renewal and the related signaling pathways. In the literature, self-renewal is generally described as a parallel cellular event of proliferation, differentiation, and apoptosis. However, accumulated evidence suggests that self-renewal of stem cells requires a combination of events: maintenance of their proliferation potential, inhibition of apoptosis, and blocking of differentiation.

Multiple signaling pathways have been reported to contribute to the regulation of stem cell self-renewal. However, different molecules and the underlying pathways may play different and overlapping roles in this regard. Maintaining proliferation potential is an obvious principle required for self-renewal of stem cells. However, it is worthwhile to point out that proliferation potential (defined as the capacity of stem cells to undergo continuous division) is different from proliferation per se in that the more the stem cells undergo active proliferation, the more they tend to lose their potential for proliferation. Therefore, stem cell proliferation potential is a functional property which can only be measured by continuous in vitro cell culture, or in vivo repopulation functional assay, rather than by measurement of the rate of proliferation. Recently, several lines of evidence have suggested that the Wnt signaling pathway through β-catenin is important for self-renewal of HSCs (Austin et al., 1997; Brandon et al., 2000; Murdoch et al., 2003; Reya et al., 2003; Van Den Berg et al., 1998; Willert et al., 2003), hair follicle stem cells (DasGupta and Fuchs, 1999; Gat et al., 1998; Huelsken et al., 2001), ISCs (He et al., 2004; Sancho et al., 2003; Sancho et al., 2004), and ESCs (Sato et al., 2004). In addition to its function in lineage fate determination, the prominent role of Wnt signaling favors cell proliferation and promotes cell growth. Abnormal activation of β-catenin leads to over-proliferation of stem cells and results in tumors in the intestines and in hair follicles (Gat et al., 1998; Sancho et al., 2004). In contrast, deletion of a Wnt downstream factor, Tcf4, leads to loss of stem cells in the intestines (Korinek et al., 1998). These observations suggest that Wnt/β-catenin signaling is important for the proliferation potential of stem cells as β-catenin may stimulate Tert (encoding the catalytic subunit of telomerase) expression via activation of Myc (He et al., 1998; Wang et al., 1998; Zou et al., 2005). The idea that limiting the proliferation potential affects stem cell self-renewal has been well demonstrated by studies of telomerase (Morrison et al., 1996), HoxB4 (Antonchuk et al., 2002; Helgason et al., 1996; Kyba et al., 2002; Sauvageau et al., 1995), p18 (Yuan et al., 2004), P21(Cheng et al., 2000), and Bmi (Lessard and Sauvageau, 2003; Molofsky et al., 2003; Park et al., 2003).

Recent reports indicate that suppression of apoptosis plays an essential role in stem cell self-renewal (Domen and Weissman, 2000; Domen et al., 2000; Opferman et al., 2005; Yamane et al., 2005). This idea is further enforced by the fact that the role of β-catenin in promoting HSC self-renewal is prominent in the Bcl2-transgenic mouse (Reya et al., 2003), indicating that a coordination between Bcl2, which inhibits apoptosis, and β-catenin, which is important for proliferation potential, is required for stem cell self-renewal. Likewise, transgenic expression of the activated form of β-catenin alone tends to lead to crypt cell apoptosis, as shown in the intestinal system (Wong et al., 1998). It is also reported that Akt is activated during intestinal stem cell activation and division (He et al., 2004), as well as during hair follicle stem cell activation (Zhang and Li manuscript, submitted). As Akt is a cell survival factor in general, activation of Akt during stem cell activation and division may be necessary to protect stem cells from apoptotic stress including that from partial anoikis, a phenomenon caused by detachment from the extracellular matrix during cell division (Khwaja et al., 1997). Consistent with this conclusion, activation of PI3K/Akt, as a consequence of the loss of PTEN-function, has been reported to result in expansion of embryonic and neural stem cell populations (Groszer et al., 2001; Kimura et al., 2003).

An important feature of stem cell self-renewal is to maintain the stem cell in an undifferentiated state. Inhibition of stem cell differentiation can lead to an accumulation of

stem cells (Chen and McKearin, 2003a,b; Shivdasani and Ingham, 2003). Therefore, some signaling pathways that function as fate determination factors to induce certain lineage commitment during development actually play a role in maintenance of stem cells by blocking stem cell differentiation under appropriate circumstances (Gat et al., 1998; Huelsken et al., 2001; Ying et al., 2003a). In this context, multiple-signal-mediated balanced control of lineage fate in stem cells can sustain the stem cell in an undifferentiated state by mutual antagonization (Ying et al., 2003a). This is exemplified by BMP signaling and its roles in regulation of stem cell self-renewal, as discussed below.

The BMP signaling pathway

BMP functions through receptor-mediated intracellular signaling and subsequently influences target gene transcription. Two types of receptors are required in this process, type I and type II. While there is only one type II BMP receptor (BmprII), there are three type I receptors: Alk2, Alk3 (Bmpr1a), and Alk6 (Bmpr1b). Different combinations of type II with any one of the type I receptors may determine the specificity and result in different consequences. There are two well-defined signaling pathways involved in BMP signal transduction (Fig. 1) (Derynck and Zhang, 2003). The canonical BMP pathway is through *r*eceptor I mediated phosphorylation of Smad1, Smad5, or Smad8 (R-Smad). Two phosphorylated R-Smads form a heterotrimeric complex with a *co*mmon Smad4 (co-Smad). The Smad heterotrimeric complex translocates into the nucleus and cooperates with other transcription factors to modulate target gene expression (Derynck and Zhang, 2003; Massague, 2000; Miyazono et al., 2000; Moustakas et al., 2001; Shi and Massague, 2003). A parallel pathway for the BMP signal is mediated by *T*GFβ1 *a*ctivated tyrosine *k*inase 1 (TAK1, a MAPKKK) and through *m*itogen *a*ctivated *p*rotein *k*inase (MAPK) (Derynck and Zhang, 2003; Massague et al., 2000; Yamaguchi et al., 1999), which also involves cross-talk between the BMP and Wnt pathways (Behrens, 2000; Ishitani et al., 2003; Ishitani et al., 1999; Smit et al., 2004; Yamaguchi et al., 1999). In addition,

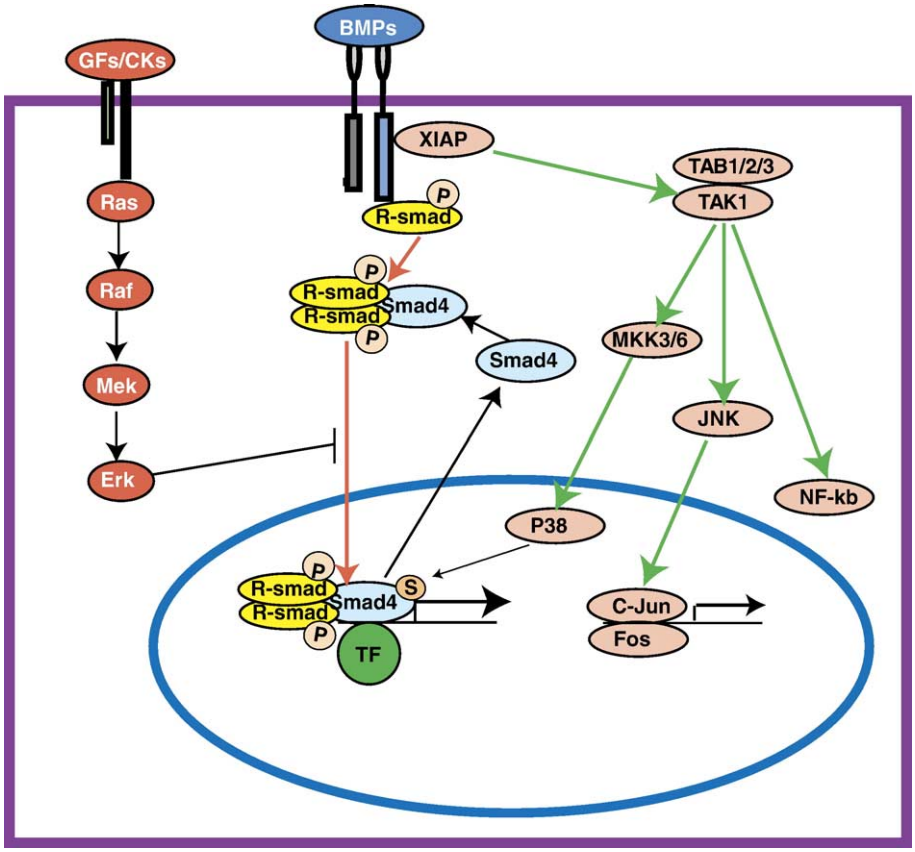

Fig. 1. BMP signal transduction pathways. Upon ligand binding, BMP type II receptor recruits type I receptor to form a complex and mediates type I receptor phosphorylation. There are at least two signaling pathways involved in BMP receptor-mediated signal transduction: Smad and TAK1/MAPK. The canonical Smad pathway is mediated by receptor-regulated R-Smad (Smad1/5/8) phosphorylation and R-Smad/Co-Smad (Smad4) complex formation. After the R-Smad/Co-Smad complex is formed, it transfers to the nucleus where it regulates target gene expression by cooperating with other transcription factors. The BMP-regulated MAPK pathway is mediated by TAK1, a MAPKKK tyrosine kinase which has multiple substrates. The mechanism of receptor-mediated TAK1 activation is still unknown. It has been reported that XIAP links the receptor to TAK1 to engage in TAK1 activation. TABs are required for fully mediated TAK1 activation. GFs/CKs induce activation of the Ras/Raf/Mek/Erk cascade. Activated Erk inhibits the Smad signal by phosphorylation of its link region and blocks Smad nuclear transfer.

X-linked *i*nhibitor of *ap*optosis (XIAP) links the BMP receptor signal to TAK1, and *TA*K1 *b*inding proteins (TAB1/2/3) are also required for BMP-mediated TAK1 activation (Massague, 2003; Yamaguchi et al., 1999). Furthermore, TAK1 can also activate *J*un *N*-terminal *k*inase (JNK) and NF-κB (Lee et al., 2002; Silverman et al., 2003; Takaesu et al., 2003).

Intriguingly, there is additional communication between the BMP signaling pathway and other *g*rowth factor/cytokine (GF/CK)-mediated signaling. Erk, in response to GF/CK signaling through Ras/Raf/Mek, can also inhibit Smad function by inhibiting Smad nuclear translocalization (Aubin et al., 2004; Kretzschmar et al., 1997a, 1999). Reciprocally, the BMP signal can also inhibit Mek/Erk activity through a yet undetermined mechanism (Qi et al., 2004).

BMP4 in ESCs—required for ESC self-renewal through a balanced inhibition of ESC lineage commitment

Embryonic stem cells (ESCs) derived from an inner cell mass (ICM) of blastocysts are pluripotent and form all types of cells in the body. ESCs can expand without losing their pluripotency in a proper in vitro culture condition (Rossant, 2004; Rossant and Tam, 2004), thus providing an attractive system for studying stem cell self-renewal and fate determination.

Mouse ESCs (mESCs) can be cultured with a layer of *m*ouse *e*mbryonic *f*ibroblast (MEF) cells which produce certain factors important for maintenance of mESCs. *L*eukemia *i*nhibitory *f*actor (LIF) is one factor produced by MEF cells that can support mESC growth (Bard and Ross, 1991; Gough et al., 1989; Smith et al., 1988; Williams et al., 1988). LIF belongs to the cytokine family that signals through cytokine receptor complexes, including a common receptor gp130, resulting in activation of transcription factor Stat3 (Boeuf et al., 1997; Burdon et al., 2002; Matsuda et al., 1999; Niwa et al., 1998; Smith, 2001). Stat3 can support mESC self-renewal as shown by over-expression of *Stat3* in culture conditions containing serum (Matsuda et al., 1999; Niwa et al., 1998; Raz et al., 1999). However, phenotypes resulting from inactivation of *Stat3* and *gp130* appear to not affect ESC self-renewal, suggesting the existence of an alternative pathway that can control ESC self-renewal as well (Smith, 2001). Intriguingly, LIF function is only effective for maintenance of mESCs in culture conditions containing serum. Furthermore, even with serum, LIF is not adequate to support self-renewal of human ESCs (hESCs) (Daheron et al., 2004; Humphrey et al., 2004; Thomson et al., 1998). In fact, LIF alone cannot maintain the pluripotency of mESCs and induces neural differentiation in a serum-free culture condition (Wilson and Edlund, 2001; Ying and Smith, 2003; Ying et al., 2003b), suggesting the existence of another factor(s) that cooperates with LIF in the maintenance of mESCs. Indeed, BMP4 was found to coordinate with LIF to maintain mESC pluripotency in a serum-free culture condition (Ying et al., 2003a).

The ability of BMP4 to maintain the pluripotency of mESCs is prominent only in coordination with LIF. A mutual and balanced inhibition between the LIF and BMP signals is critical for the maintenance of ESCs (Fig. 2). The

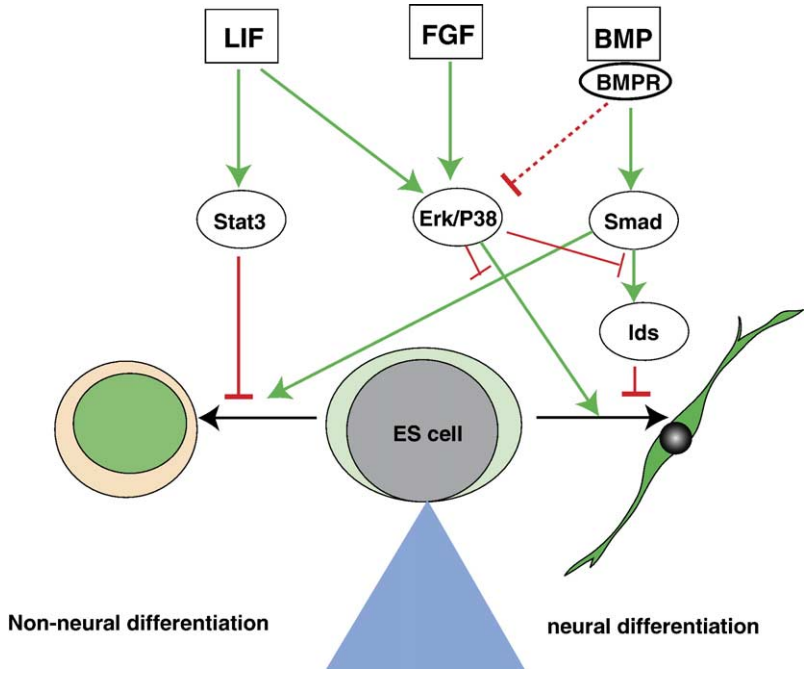

Fig. 2. BMP blocks ES cell neural differentiation and maintains ES cell pluripotency by forming a balance with growth factor (LIF and FGF) signaling. The BMP signal inhibits growth factor/MAPK signal-mediated neural differentiation and induces non-neural differentiation. LIF and FGF block BMP-stimulated non-neural differentiation and cooperate with BMP in ES cell self-renewal regulation through the Erk/P38 MAPK pathway or LIF/Stat3 signaling.

BMP signal is well known for its function to inhibit neural differentiation in both embryos (Wilson and Edlund, 2001) and ESCs (Tropepe et al., 2001; Ying et al., 2003a), as well as to induce the lineage fates of mesoderm, endoderm, and trophoblast (Li et al., 2001; Nakayama et al., 2000; Pera et al., 2004; Xu et al., 2002; Ying et al., 2003a). In contrast, LIF through Stat3 can block mesoderm and endoderm differentiation but favors neural differentiation (Ying et al., 2003a). This is supported by the observation that over-expression of Smad1/4 or introduction of the constitutively active BMP receptor into mESCs, overrides the effects of LIF and causes non-neural differentiation (Ying et al., 2003a). The inability to generate mESCs from *Bmpr1a*$^{-/-}$ blastocysts indicates that Bmpr1a is involved in the BMP4-LIF balanced control of ESCs (Qi et al., 2004).

Id2 expression controlled by Smad-mediated transcriptional regulation has been shown to be involved in inhibition of ESC neural differentiation (Ying et al., 2003a). The BMP function in maintaining mESC cannot be replaced by TGFβ. This is due to Smad1-, 5-, or 8-dependent regulation of *Id2* expression by BMPs but not by TGFβ. Over-expression of Id2 can replace BMP function in inhibition of neural differentiation, and its induction role in non-neural differentiation is blocked by LIF (Ying et al., 2003a). However, the ability to establish mESCs from Smad4$^{-/-}$ blastocysts argues that an alternative BMP signaling pathway may also be involved in maintenance of ESCs (Qi et al., 2004).

Unlike mESCs, which require LIF to support their self-renewal, human (h) ESCs require *b*asic *f*ibroblast *f*actor (bFGF) to maintain their self-renewal when cultured with a layer of feeder cells or fibroblast-conditioned medium (Amit et al., 2000; Thomson et al., 1998). Recently, it was found that hESCs have a high level of BMP activity in unconditioned culture medium (without feeder cells or fibroblast-conditioned medium). A higher level of bFGF alone, or lower bFGF in combination with the BMP antagonist Noggin, is required to reduce the BMP activity and thus supports the long-term undifferentiated proliferation of hESCs in this culture condition (Xu et al., 2005). Furthermore, signaling by either LIF or bFGF through Erk and P38 MAP kinase favors neural differentiation and inhibits non-neural differentiation (Fig. 2). This result again indicates that balanced inhibition of ESC lineage commitment is essential to maintain stem cells.

Erk and P38 MAP kinase, which are subject to regulation by either LIF or FGF signaling pathways, play critical roles in induction of ES cell differentiation (Burdon et al., 1999a,b, 2002). FGF signaling through activation of Mek/Erk induces mESC neural differentiation (Ying and Smith, 2003; Ying et al., 2003b). It has also been reported that enhancement of ES cell self-renewal by the phosphatidylinosital-3 kinase (PI3k)/Akt (a serine/threonine kinase) signaling pathway is through inhibition of Erk and P38 MAP kinase activity and the subsequent differentiation (Paling et al., 2004). Therefore, the balance between BMP and LIF signals in maintenance of mESCs is also at least partially due to inhibition of Erk and P38 MAP kinase pathways (Qi et al., 2004). BMP signaling represses activation of Erk and P38 MAP kinase and thereby inhibits neural differentiation. Thus, blocking Erk/P38 activity can replace the combined functions of BMP and PI3k/Akt in the maintenance of ESC pluripotency (Paling et al., 2004; Qi et al., 2004) (Fig. 2).

Although the molecular mechanism by which the BMP signal inhibits Erk and P38 MAP kinase needs further investigation, a study of ESCs derived from Bmpr1a$^{-/-}$ mouse suggests that Bmpr1a is required for suppression of Erk/P38 activity; otherwise, ESCs cannot be established from Bmpr1a$^{-/-}$ mouse blastocysts without inhibition of P38 kinase activity (Qi et al., 2004). Thus, both Smad pathway activation and MAPK pathway inhibition are involved in BMP-mediated maintenance of ESCs. It is important to investigate how these two pathways coordinate in this regard. Smad proteins, important BMP signaling transducers, have two conserved globular domains—MH1 and MH2. A linker region with variable sequence and length lies between the MH1 and MH2 domains. The activity of Smad proteins is regulated through phosphorylation at different sites by different kinases (Massague, 2003). BMP receptor-mediated Smad phosphorylation occurs at the carboxy-terminal sequence SXS, which leads to signal activation (Shi and Massague, 2003). Recently, it has become clear that FGF signaling through the MEK/Erk pathway can inhibit Smad activation through phosphorylation of the link region of Smad1 (Aubin et al., 2004; Kretzschmar et al., 1997a,b, 1999). Reciprocally, Erk/P38 activity can be inhibited by the BMP-TAK1 cascade (Goswami et al., 2001; Qi et al., 2004), and possibly through the Smad-Id pathway as well. Regardless, the mutually antagonistic interaction between BMP and Erk MAPK signaling pathways is important for ESC self-renewal and maintenance. The fact that multiple signals are required for a balanced control of ESCs, inhibiting both neural and mesoderm differentiation, strongly supports the idea that inhibition of differentiation is required for self-renewal of stem cells.

The role of BMP signaling in maintenance of germline stem cells in *Drosophila* through inhibition of bam-mediated differentiation

Germline stem cells (GSCs) located at the tip of the germarium in the *Drosophila* ovary provide another premier system for studying stem cell self-renewal and fate determination. Cap cells adjacent to GSCs, together with terminal filament cells, form the niche supporting GSCs (Lin, 2002; Xie and Spradling, 2000). Signals generated from the niche, including BMP, regulate the proper behavior of GSCs (Lin, 2002; Spradling et al., 2001).

BMPs generated by cap cells include Dpp and *G*lass *b*ottom *b*oat (Gbb) and have been shown to play a role in regulation of GSC self-renewal. This is evident by the fact that over-expression of *Dpp* results in uncontrolled expansion of GSCs, while disruption of Dpp/Gbb signaling by deletion of either gene leads to loss of GSCs and germ cells (Lin, 2002; Song et al., 2004; Xie and Spradling, 1998, 2000). Recent studies from several groups indicate that the role of Dpp/Gbb in maintenance of GSCs is mainly attributable to its ability to repress transcription of *ba*g of *m*arbles (*bam*), a differentiation-promoting gene (Casanueva and Ferguson, 2004; Chen and McKearin, 2003a,b; Song et al., 2004). Dpp/Gbb is proposed as a short-region signal emanating from the niche cells, which mainly inhibits *bam* expression in GSCs, thereby preventing stem cell differentiation. In contrast, expression of *bam* is exclusive in cytoblasts (the progeny of stem cells) and cyst cells, thus leading to cell differentiation.

A similar phenomenon is also seen in the *Drosophila* testis where Hub cells, instead of cap cells, function as the niche to support GSCs (Kiger et al., 2001; Tulina and Matunis, 2001; Yamashita et al., 2003). Dpp/Gbb signaling is active in GSCs and gonialblasts, the immediate daughter cells of GSCs, but is inactive in other differentiated germ cells. Disruption of the Dpp/Gbb signaling pathway results in GSC loss (Kawase et al., 2004; Shivdasani and Ingham, 2003). Dpp and Gbb cooperate in their function to maintain GSCs in the *Drosophila* testis (Kawase et al., 2004). The role of Dpp/Gpp signaling in maintenance of male GSCs also occurs through inhibition of *bam* expression, which controls GSC and gonialblast differentiation (Kawase et al., 2004; Shivdasani and Ingham, 2003), although the phenotype is not as dramatic as that observed in female ovary. This suggests that alternative pathways are also involved in male GSC regulation. Indeed, in male GSCs, unpaired (Upd), a Hub cell-secreted factor, activated JAK/STAT signaling plays a critical role in GSC self-renewal (Kiger et al., 2001; Tulina and Matunis, 2001).

In summary, the role of Dpp/Gbb in the maintenance of GSCs in *Drosophila* is consistent with the role of BMP4 in ESCs through inhibition of cell differentiation (Fig. 3). These observations again support the argument that suppression of differentiation is an important mechanism for stem cell self-renewal. Recently, Nanos was shown to be required for GSC self-renewal through suppression of differentiation (Wang and Lin, 2004). As Nanos is required for general translational regulation, this observation reinforces the idea that suppression of differentiation is important for stem cell self-renewal.

The importance of BMP signaling in regulation of GSCs is also conserved in mammals. For example, BMP4 and BMP8b (a member of the Gbb-60A class of the BMP superfamily) have been shown to play critical roles in the induction of mouse primordial germ cells (PGC) during early embryonic development (Lawson et al., 1999; Ying et al., 2000). BMP4 and BMP8b enhance PGC production from the embryonic body (Toyooka et al., 2003) and from the epiblast in in vitro culture (Hayashi et al., 2002; Okamura et al., 2005; Pesce et al., 2002; Ying et al., 2001). On the other hand, mice with a BMP4 or BMP8b mutation lack PGC formation (Lawson et al., 1999; Ying et al., 2000, 2001). Furthermore, PGC induction produced by BMP4 and BMP8b depends on receptor Alk2 and transcription factors Smad1 and Smad5 (Chang and Matzuk, 2001; de Sousa Lopes et al., 2004; Hayashi et al., 2002; Tremblay et al., 2001).

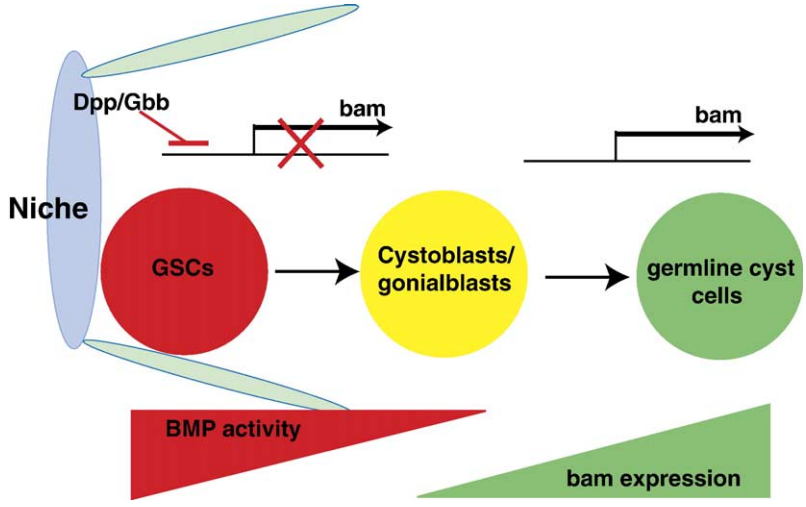

Fig. 3. BMP signaling maintains GSC stemness by blocking differentiation in the *Drosophila* ovary and testis. GSC niche cell secreted Dpp/Gbb forms a gradient. This Dpp/Gbb gradient mediates a short region of BMP activity that represses bam transcription in GSCs and cystoblasts/gonialblasts. In germline cyst cells, there is no detectable BMP activity and *bam* expression is released from BMP inhibition. Bam plays important roles in GSC differentiation. Disruption of bam functioning or inhibition of *bam* expression leads to GSC accumulation, even GSC-like tumor formation.

BMPs and hematopoietic stem cell regulation—the niche size controls stem cell number

The hematopoietic system serves as the pioneer paradigm for studying stem cells (Schofield, 1978; Spangrude et al., 1988; Till and McCulloch, 1961). Hematopoietic tissue is derived from embryonic mesoderm and the location of hematopoiesis dynamically changes at the different developmental stages: from yolk sac to *A*orta *G*onad *M*esonephros (AGM) (Dzierzak, 1999; Dzierzak and Medvinsky, 1995), to placenta (Gekas et al., 2005; Ottersbach and Dzierzak, 2005), to fetal liver (Ikuta et al., 1990; Jordan et al., 1990), and finally to bone marrow after birth (Dzierzak, 1999, 2003; Dzierzak and Medvinsky, 1995). BMPs have been shown to play important roles in induction of hematopoietic tissue during early embryonic development (Dzierzak, 2003; Maeno et al., 1996). BMP4 has also been shown to be able to maintain the reconstitution ability of HSCs under in vitro culture conditions (Bhatia et al., 1999). The in vivo function of BMP signaling in the regulation of HSCs has been elusive until recently. In our analysis of a conditional *Bmpr1a* mutant mouse model, we found that the pool size of HSCs is controlled by the volume of trabecular bone (Zhang et al., 2003); the larger the volume, the greater the HSC number. This observation is consistent with a simultaneous study using a transgenic mouse model in which parathyroid hormone receptor (PTHR) is expressed (Calvi et al., 2003). These two studies led to the identification of a subset of osteoblastic cells (N-cadherin+) lining the bone surface as the niche supporting HSCs. In the hematopoietic niche, N-cadherin and β-catenin form an adhesion complex at the interface between HSCs and osteoblastic cells (Zhang et al., 2003), facilitating HSC anchoring to the niche. Jagged–Notch interaction is also important for the maintenance of HSCs (Calvi et al., 2003; Duncan et al., 2005). In this study, we provided solid evidence to support the conclusion that BMP signaling mediated by Bmpr1a plays a role in controlling the HSC number through regulation of the niche size (Zhang et al., 2003) (Fig. 4).

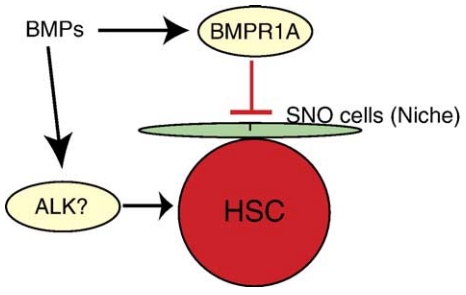

Fig. 4. BMP signaling through BMPR1A indirectly regulates HSCs by controlling the HSC niche size. The SNO cell represents the *s*pindle-*s*haped *o*steoblast, one of the key components of the bone marrow HSC niche (Zhang et al., 2003). The direct roles of BMP in HSC regulation are yet to be determined.

Recently, by studying BMP signaling in the intestinal stem cell compartment, we found that BMP signaling also plays a direct role in restriction of stem cell activation and expansion through suppression of Wnt/β-catenin activity (for details, please see He et al., 2004). This BMP-Wnt antagonism has also been seen in different systems and by different groups (Haramis et al., 2004; Jamora et al., 2003).

Closing remarks

In this review, we have compared the different roles that BMP signaling plays in different stem cell compartments. The data from ESC studies indicate that the balance between BMP and LIF/bFGF signaling, for mutual inhibition of neural and mesoderm/endoderm differentiation, respectively, is critical for ESC maintenance. This mechanism may also hold true in some adult stem cells, including germline stem cells. Indeed, the role of Dpp/Gbb in maintenance of GSCs in *Drosophila* is through inhibition of bam-mediated cell differentiation. The difference is that *Drosophila* GSCs can only give rise to a single lineage—cystoblast in the ovary or gonialblast in testis. Blocking differentiation tends to result in an accumulation of GSCs, while in most tissue stem cells with multipotentiality, blocking one lineage fate often drives the cells to alternative lineages, and may not result in accumulation of stem cells. Therefore, coordination between at least two or more signals is required to maintain a balanced control of stem cell differentiation, insuring the maintenance of stem cells in their undifferentiated state. All of these indicate that differentiation inhibition is an important mechanism for stem cell self-renewal.

The study of *Bmpr1a* mutant mice in HSC regulation indicates that the influence of BMP signaling on stem cells can be direct or indirect. Stem cells need a homeostatic niche for proper regulation. Alterations in the stem cell population could be due to the secondary effect of microenvironmental change, emphasizing the complicated network within stem cells, signaling between niche and stem cells, and circulating signals, all of which can impose additional layers of regulation on stem cell behavior. The study of different stem cell systems provides a complementary view regarding the role of BMP signaling in regulation of stem cell properties, and also provides important insight into understanding the principles used by stem cells in self-renewal, including at least three elements: blocking differentiation, suppressing apoptosis, and maintaining proliferation potential.

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