Role of the bone morphogenic protein pathway in developmental haemopoiesis and leukaemogenesis

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Abstract

Myeloid leukaemia's share the common characteristics of being stem cell-derived clonal diseases, characterised by excessive proliferation of one or more myeloid lineage. Chronic myeloid leukaemia (CML) arises from a genetic alteration in a normal haemopoietic stem cell (HSC) giving rise to a leukaemic stem cell (LSC) within the bone marrow (BM) 'niche'. CML is characterised by the presence of the oncogenic tyrosine kinase fusion protein BCR-ABL, which is responsible for driving the disease through activation of downstream signal transduction pathways. Recent evidence from our group and others indicates that important regulatory networks involved in establishing primitive and definitive haemopoiesis during development are reactivated in myeloid leukaemia, giving rise to an LSC population with altered self-renewal and differentiation properties. In this review we explore the role BMP signalling plays in stem cell pluripotency, developmental haemopoiesis, HSC maintenance and the implication of altered BMP signalling on LSC persistence in the BM niche. Overall we highlight how the BMP and Wnt pathways converge to alter the Cdx-Hox axis and the implications of this in the pathogenesis of myeloid malignancies.

Key words: Haemopoiesis, Chronic myeloid leukaemia (CML), leukaemic stem cell (LSC), bone marrow (BM) niche, bone morphogenic protein (BMP) pathway, Cdx-Hox

Abbreviations: Acute myeloid leukaemia (AML), Adenomatous polyposis coli (APC), All-trans retinoic acid (ATRA), Anaplastic lymphoma kinase (ALK), Anti Mullerian Hormone (AMH), Aorta-gonad-mesonephros (AGM), Blast colony forming cells (BL-CFCs), Bone marrow (BM), Bone morphogenic protein (BMP), Bone morphogenic protein type II receptor (BMPRII), Cancer stem cell (CSC), Chronic myeloid leukaemia (CML), Embryoid bodies (EBs), Dishevelled (DVL), Extracellular signal-regulated kinases (ERK), Fibroblast growth factor (FGF), Fibroblast growth factor receptor (FGFR), Follistatin-related gene (FLRG), Frizzled (FZD), Fusion Regulatory Protein (FRP), green fluorescent protein (GFP), Glycogen synthase kinase 3 (GSK3), Haemopoietic stem cells (HSCs), High-proliferative potential colony forming cells (HPP-CFCs), Human embryonic stem cells (hESCs), Hedgehog (Hh), Inhibitor of differentiation (Id), Insulin-like growth factor (IGF), Janus N-terminal kinase (JNK), Leukaemia inhibitory factor (LIF), Leukaemic stem cells (LSCs), Low Density Lipoprotein Receptor-Related Protein (LRP), Lymphoid enhancing factor (LEF), MAPK/Erk kinase (MEK), Mitogen-activated protein kinase (MAPK), Mixed lineage leukaemia (MLL), Murine embryonic stem cells (mESCs), Myeloid ecotropic viral integration site 1 (MEIS1), Para-aortic splanchnopleure (P-Sp), Phosphatidylinositol-3 kinase (PI3K), Promyelocytic leukaemia (APL), Promyelocytic leukaemia

(PML), Transforming growth factor-β (TGFβ), Vascular Endothelial Growth Factor (VEGF), Wingless-type MMTV integration site family member (Wnt)

Funding: This work was supported by a Ph.D. studentship from the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs).

The bone morphogenic protein (BMP) pathway

The bone morphogenic protein (BMP) ligands are part of the transforming growth factor-β (TGFβ) superfamily, which includes TGFβs, Activins/Inhibins, Nodal, Myostatin, and Anti Mullerian Hormone (AMH). BMP ligands possess different affinities for the BMP receptors, which determine their specificity of action [1, 2]. Activation of the BMP pathway occurs either in a canonical SMAD-dependant or SMAD-independent manner. The former is dependent on SMAD proteins for regulation whereas the latter involves extracellular signal-regulated kinases (ERK), Janus N-terminal kinase (JNK) and p38 Mitogen-activated protein kinase (MAPK) pathways. The canonical BMP pathway relies on a series of signal transduction steps which is initiated when BMP ligands bind to type II receptors. Ligand binding then leads to the heterodimerization of the type I and type II receptors. This in turn phosphorylates the corresponding receptor regulated SMADs (R-SMADs) and drives the formation of an R-SMAD-Co-SMAD complex. This complex translocates to the nucleus where it binds to relevant transcription factors/initiators resulting in the transcription of BMP target genes involved in the cell cycle, proliferation and self-renewal [1, 2].

The role of BMP pathway in the maintenance of pluripotency

Human and mouse embryonic stem cells (ESCs) maintain their pluripotency through the expression of a complex transcription factor network with the key transcription factors being; Oct4, Nanog, Sox2, Klf4, Rex1 and Cited2 [3]. Several signalling pathways co-ordinate this transcriptional network, with the TGFβ superfamily signalling pathway contributing to the maintenance of pluripotency and early differentiation decisions [4]. Transcriptome analyses of human ESCs (hESCs) cultured on feeder cells revealed these cells require signals mediated by fibroblast growth factor 2 (FGF2), activin, TGFβ, insulin, and insulin-like growth factor (IGF) to maintain their undifferentiated state [5]. In hESCs TGFβ and FGF2 cooperate to inhibit BMP signalling and sustain the expression of pluripotency markers, especially via activated SMAD3 binding to the NANOG promoter. Activation of SMAD2/3 signals is required as a downstream event to maintain the undifferentiated state of hESCs [4, 6]. On the other hand the proximal NANOG promoter contains the TGFβ/activin responsive element, which is negatively regulated by signals from the BMP pathway. Xu *et al.* demonstrated that culturing hESCs in high amounts of FGF2 in combination with inhibiting the BMP pathway using the BMP antagonist noggin (NOG) maintains pluripotency and long term, undifferentiated proliferation of hESCs [4, 7]. FGF2 maintains hESCs by activating the MAPK pathways while the signals mediated by Activin/Nodal and IGF maintain pluripotency via activation of Ras and phosphatidylinositol-3 kinase (PI3K) pathways [4, 7] (Figure 1).

In murine ES cells (mESCs) self-renewal capacity can be maintained by the cytokine leukaemia inhibitory factor (LIF) [8]. However, the presence of LIF is not totally sufficient as the cells also require the presence of foetal calf serum to maintain pluripotency. It was shown that the BMPs present in the serum are the key additional signalling molecules which act in conjunction with LIF, enhancing the self-renewal and pluripotency of mESCs [4, 9]. Further work revealed that activation of the BMP pathway through BMP4 stimulation leads to the expression of inhibitor of differentiation gene 1 (Id1), critical for suppressing ESC differentiation and sustaining pluripotency [9]. This is supported by other studies showing that overexpression of Id1 encourages mESCs to self-renew in the absence of BMP4, with suppression of Id1 leading to decreased Nanog expression, which results in the failure of mESCs to maintain self-renewal [10].

BMP pathway and the initiation and maintenance of embryonic haemopoiesis

BMP signalling plays a key role during early embryogenesis and subsequent organogenesis. The BMP and Wnt pathways are essential for mesodermal patterning and subsequent specification of haemopoiesis in the developing embryo [11]. Haemopoietic and endothelial cells are among the earliest cells to form in the embryo, with BMP pathway involvement in the initiation of this process conserved throughout evolution [11, 12]. These cells arise during gastrulation from mesodermal progenitors in the epiblast [13]. Studies using targeted gene disruption in murine models revealed BMP2 [14], BMP4 [15], anaplastic lymphoma kinase 3 (ALK3) [12] and BMP type II receptor (BMPRII) [16] play important roles in early haemopoiesis as all these defects cause early embryonic lethality and reduced formation of mesoderm. In particular, BMP4 plays a key role in mesoderm induction and haemopoietic commitment, and is fundamental for inducing haemopoietic differentiation in murine [17] and human [18] ESCs *in vitro*. In mESCs BMP4 induces the formation of mesoderm and early haemopoietic precursors whereas inhibition of BMP signalling impaired haemopoietic development [17, 19]. In hESCs, BMP4 in combination with cytokines also enhanced haemopoietic differentiation [18]. Developmental studies indicate that the BMP and Wnt pathways converge to regulate the Cdx family of homeobox transcription factors, master regulators of Hox gene expression [20, 21]. Activation of BMP pathway induces Wnt3a and thereby the canonical Wnt pathway, which in turn activates the Cdx-Hox axis [21]. Furthermore in mESCs overexpression of Cdx1 and Cdx4 induces Wnt3a suggesting that Wnt signalling also acts downstream of Cdx proteins [21]. Studies from zebra fish and mESCs indicate that Cdx-driven patterning is necessary for the development of mesoderm derivatives such as the haemopoietic system [20, 21]. During early development Cdx genes show a similar expression pattern to Hox genes. In mice, this expression is detected in the posterior epiblast and the overlaying mesoderm at the posterior end of the primitive streak. As reviewed by Lengerke and Daley during the development in the posterior trunk zone, anterior trunk tissues are exposed to Cdx genes but as cells move anteriorly, Cdx transcripts decrease [20]. Cdx genes expression persists in the posterior region of the embryo. This is then followed by the expression of more posterior Hox genes which enables the development of posterior trunk mesoderm and tail. Cdx4-deficient mice have been shown to produce reduced numbers of yolk sac-derived erythroid colonies [20].

Primitive haemopoiesis during murine development takes place in the yolk sac between E7 and E11, whereas in the human system primitive erythropoiesis is initiated in the yolk sac around week 3 of gestation and remains active until week 6. During mammalian development, BMP4 is involved in the induction of the yolk sac haemopoietic program. It has been shown that signals from the visceral endoderm, such as Indian hedgehog (Hh), are critical for the development of the yolk sac haemopoietic program and the regulation of BMP4 expression [22]. Mouse studies illustrate BMP4 knockout mice die between E7.5 and E9.5 exhibiting severe defects in mesoderm formation and the embryos that survive up to E9.5 show defective blood islands [15]. Studies on other components of BMP pathway further highlight the role of this family in haemopoietic development. Disruption of the Smad5 gene in mice leads to severe defects in yolk sac circulation and subsequent death around mid-gestation [20]. Yolk sacs from these embryos ($Smad5^{-/-}$ E9.0 to 9.5 yolk sac) have increased numbers of high-proliferative potential colony forming cells (HPP-CFCs) with enhanced re-plating potential. These data suggest that Smad5 transduces signals that inhibit the early specification events of haemopoietic precursors as well as their subsequent expansion [21].

Primitive yolk sac haemopoiesis is then replaced by the definitive, HSCs derived multi-lineage blood system that is sustained throughout life [23]. The area of the embryo known as the para-aortic splanchnopleura (P-Sp), E8.5-9.5 in the mouse, is the first site to produce haemopoietic progenitors with myeloid and lymphoid potential [24], however these cells are unable to sustain long term reconstitution of irradiated recipients [25]. The P-Sp gives rise to the developing aorta, gonads and mesonephros (AGM) region by E10-11.5 [26]. The AGM is the site where the first definitive HSCs arise, these emerge as clusters of cells from the ventral wall of the dorsal aorta from E10.5, these cells are multipotent and display long term reconstitution potential [27-29]. In mouse and human embryos, BMP4 is expressed in the mesenchyme underlying the aorta and is required for HSC maintenance [30-32]. It has been shown using BMP responsive element (*BRE*) green fluorescent protein (*GFP)* transgenic mouse embryos that the first HSCs emerging *in vivo* in the AGM region are GFP^{+} i.e. BMP activated and have long term reconstituting potential. However the proportion of BMP activated HSCs decreases through ontogeny, with approximately three quarters of the HSCs in the foetal liver (FL) being GFP^{+} BMP activated whereas the majority of HSCs found in the bone marrow (BM) are GFP^{-} i.e. non-BMP activated. The two types of HSCs found in the FL and BM display distinct differences in their lineage output and their intrinsic molecular programs, with BMP activated HSCs favouring a more myeloid-lymphoid balanced differentiation programme [33]. Explant studies using E11 AGM have revealed that over the 3 day culture period GFP^{-} non-BMP activated HSCs emerge and these cells are controlled by the Hh/Vascular Endothelial Growth Factor (VEGF) pathway. Elegant transplantation experiments and transcriptome data indicate that the GFP^{+} BMP activated HSCs can give rise to the GFP^{-} Hh/VEGF responsive HSCs, with the GFP^{+} expressing a more haemogenic endothelial molecular program, whereas the GFP^{-} express a haemopoietic program [34]. As in the mouse, the AGM region in humans contains definitive haemopoietic cells prior to their detection in the FL [35]. Between E12-13 in the mouse, the HSCs migrate and populate the developing FL, which becomes the principal site of haemopoiesis throughout the remainder of foetal life. Whereas in humans the FL continues as a major site of haemopoiesis until week 22, by which

time the foetal bones, which start to become colonised by HSCs in weeks 8–12, then takes over as the major site of haemopoiesis [35]. By birth the BM is established as the major site of haemopoiesis and continues as the major source of haemopoietic cells throughout life. In adults the embryonic morphogens including the TGFβ superfamily play an important role in maintaining haemopoietic homeostasis. Osteoblasts, stromal cells, megakaryocytes and platelets all secrete BMPs, which have been implicated in the self-renewal and maintenance of HSCs, as well as regulating the expansion of progenitor cells and their differentiation into the mature lineages of the haemopoietic system [36] (Figure 2).

Reactivation of embryonic pathways in leukaemia

HSCs have three key properties of long life, multipotency and self-renewal. Within the BM, control of steady state haemopoiesis is regulated by extrinsic and intrinsic factors which control the balance between HSC self-renewal and differentiation. Cell extrinsic cues are governed by the stem cell niche and include growth factors, cytokines, chemokines, oxygen tension, nutrients and cell-to-cell interactions with stroma, endothelium, osteoblasts and the extra-cellular matrix. These signals merge into a network of intrinsic regulators resulting in the activation of signalling pathways, induction of transcription factors and changes to epigenetic marks [37, 38]. All of which govern the balance between self-renewal and differentiation of HSCs. Recent evidence indicates that important regulatory networks involved in establishing primitive and definitive haemopoiesis during development are reactivated in leukaemias, giving rise to a LSC population with altered self-renewal and differentiation properties. In particular altered activation of the conserved embryonic morphogenic pathways including; Wnt, Hedgehog, BMP, TGFβ, and Notch as well as the Cdx-Hox transcription factors and the BMI1/ polycomb transcriptional regulators is becoming an emerging theme in myeloid leukaemia [37]. In mixed lineage leukaemia (MLL) it has been shown that the LSCs are maintained in a self-renewing state through expression of a transcription program more akin to ESCs, than adult stem cells and that the LSCs occupy the apex of a leukaemia cell hierarchy, supporting the cancer stem cell model of disease [39].

Role of BMP pathway in leukaemia and leukaemic stem cells (LSCs)

BMP ligands are naturally produced and secreted by cells in the BM microenvironment [36]. There are more than 500 genes controlled by BMP and TGFβ pathways, which are involved in HSC maintenance and self-renewal [40]. TGFβ signalling is important for HSC quiescence through its anti-proliferative properties, as well as immune cell homeostasis and its ability to decrease meylopoiesis. BMPs are known to regulate HSCs self-renewal and differentiation through intrinsic and extrinsic mechanisms [41]. BMP2, BMP4, BMP7 and Smad proteins have been implicated in the self-renewal and maintenance of HSCs, expansion of progenitor cells and their differentiation into the mature lineages of the haemopoietic system [42, 43]. In normal haemopoiesis BMP2, BMP4 and BMP7 regulate proliferation, maintenance, clonogenicity and repopulating activities of immature blood progenitors [44].

Given the important roles BMPs play in stem cell biology and haemopoiesis it is surprising that deregulation of this pathway has only been shown to occur in two types of leukaemia to date; namely acute promyelocytic leukaemia (APL) [45] and CML [36, 46]. However recent research has started to focus on the leukaemic BM niche, especially the leukaemic endosteal niche. In many myeloid leukaemias there is enhanced osteoblastic proliferation and a marked increase in LSCs and progenitor expansion [37, 46]. LSCs strongly rely on the BM niche for their self-renewal and proliferation and may also modify it to their advantage. Recent research on the role of the microenvironment in CML confirms elevated levels of BMPs in the BM of chronic phase (CP) patients [46]. This study illustrates that deregulation of the BMP pathway may lead to HSC expansion due to exposure of $CD34^{+}$ cells which overexpress BMPR1B (ALK6) to the increased amount of soluble BMP2/ BMP4 in the BM [46]. Moreover transcriptome studies of CML LSCs and progenitors indicate that the TGFβ and BMP pathways are downregulated in CP CML which suggests an extrinsic mechanism for TGFβ involvement in this disease [46]. Deregulation of this pathway has also been shown in murine models where Bmpr1a/Alk3 conditional knockout mouse have impaired BMP signalling, which leads to increased niche size and thereby enhanced numbers of HSCs [47].

Although the BMP pathway hasn't been directly linked to many myeloid malignancies, its downstream target genes, the Cdx-Hox axis are an emerging theme in leukaemia (Figure 3). It is known that Wnt and BMP signalling converge to regulate the Cdx family of homeobox transcription factors, master regulators of Hox gene expression [21, 48]. Wnt/GSK3β/-βcatenin signalling, which plays an important role in HSC homeostasis is altered in blast-crisis CML [37, 49]. Increased levels of active β-catenin and components of the Wnt pathway have also been shown to occur in AML patients, with the enhanced re-plating capacity of these LSCs abrogated by inhibiting Wnt signalling [37, 50]. Consensus binding sites for the three Cdx homologues Cdx1, Cdx2, and Cdx4 are present in the promoters of multiple HOX genes [51]. Cdx2 is aberrantly expressed in AML and promotes leukemogenesis via deregulation of Hox genes [52]. The study by Scholl *et al.* indicated that CDX2 is overexpressed in 90% of patients with AML [52]. In this study, overexpression of Cdx2 in primary murine haemopoietic progenitors resulted in transplantable AML *in vivo*, coinciding with a three-fold up regulation in Hoxb6 expression. Interestingly, overexpression of HOXB6 has been documented in approximately 40% of human AMLs that do not have chromosomal translocations [53]. Furthermore other family members of CDX2 such as CDX1, and CDX4 are direct targets of the Wnt pathway and are up regulated in AML [52].

Substantial evidence has now linked aberrant expression of HOX genes to the pathogenesis of myeloid malignancies, especially AML and CML [54, 55]. HOX genes have been reported to be deregulated in AML through several different mechanisms. Fusions of the HOXA9 or HOXD13 genes with NUP98, a gene that encodes a component of the nuclear pore complex, have been described in AML, and recapitulates AML in murine models of disease [55-57]. Furthermore overexpression of HOXA6, HOXA7, HOXA9, and the HOX co-factor MEIS1 has also been correlated with chromosome 11q23 abnormalities involving the MLL protein, which regulates the expression of HOX genes [58]. The t(8;16)(p11;p13) translocation in AML, results in the expression of the MYST3-CREBBP fusion

protein and the up regulation of HOXA9, HOXB9, HOXA10, and MEIS1 [59]. In addition, the expression of HOXC4 was shown to be up regulated in the NB4 PML-RARα cell line following all-trans retinoic acid–induced (ATRA-induced) differentiation as well as in BM from acute promyelocytic leukaemia (PML) patients during ATRA treatment [60]. Over expression of individual Hox family members, including HoxB3 [61], HoxB8 [62], or HoxA10 [61], by retroviral expression or retroviral insertion mutagenesis also generates AML in murine models. In addition up regulation of specific Hox genes, such as HoxB4 or HoxA9, is associated with expansion of the HSC compartment *in vitro* and *in vivo* and results in enhanced competitive repopulating activity in murine transplantation experiments [63, 64]. Changes in Hox gene expression patterns have also been linked to clinical variables in myeloid leukaemias, most notably in CML, where hypermethylation of HOXA5 and HOXA4 strongly correlates with progression to blast crisis [65]. Considering both the BMP and Wnt signalling pathways are known to be deregulated in CML, it is feasible to speculate that the elevated BMP ligands observed in the BM of the CML patients [46] may alter the Cdx-Hox gene axis resulting in an altered balance in LSCs self-renewal and myelopoiesis.

Overall these data demonstrate the BMP signalling pathway plays a fundamental role during embryogenesis and haemopoietic ontogeny. This family of morphogens act at multiple levels during development, starting at the earliest stage by influencing pluripotent stem cell self-renewal, followed by mesodermal specification, then later on during the establishment of primitive and definitive haemopoiesis in the embryo and finally playing a role in haemopoietic equilibrium in the adult. Evidence from the literature indicates that BMP activation of HSCs during AGM emergence in the murine setting may sustain their myeloid-lymphoid differentiation potential as they migrate and expand within different anatomical sites during development. Interestingly by the time the HSCs populate the BM the majority are no longer BMP activated, which could potentially reflect cell autologous changes or the influence of the different extrinsic signals within the FL and BM microenvironments. The influence of BMP signalling on lineage specification has important implications for leukaemia and may enhance our understanding in the future as to why paediatric leukaemia is predominantly lymphoid whereas adult leukaemia is predominantly myeloid. Future studies will unravel the complexities of how altered BMP signalling within the malignant haemopoietic niche perturbs the equilibrium to aid aberrant haemopoiesis and the development of disease.

References

1 Herpin, A. and Cunningham, C. (2007) Cross-talk between the bone morphogenetic protein pathway and other major signaling pathways results in tightly regulated cell-specific outcomes. The FEBS journal. **274**, 2977-2985

2 Miyazono, K., Maeda, S. and Imamura, T. (2005) BMP receptor signaling: transcriptional targets, regulation of signals, and signaling cross-talk. Cytokine & growth factor reviews. **16**, 251-263

3 Boyer, L. A., Lee, T. I., Cole, M. F., Johnstone, S. E., Levine, S. S., Zucker, J. P., Guenther, M. G., Kumar, R. M., Murray, H. L., Jenner, R. G., Gifford, D. K., Melton, D. A., Jaenisch, R. and Young, R. A. (2005) Core transcriptional regulatory circuitry in human embryonic stem cells. Cell. **122**, 947-956

4 Itoh, F., Watabe, T. and Miyazono, K. (2014) Roles of TGF-beta family signals in the fate determination of pluripotent stem cells. Seminars in cell & developmental biology. **32**, 98-106

5 Wei, C. L., Miura, T., Robson, P., Lim, S. K., Xu, X. Q., Lee, M. Y., Gupta, S., Stanton, L., Luo, Y., Schmitt, J., Thies, S., Wang, W., Khrebtukova, I., Zhou, D., Liu, E. T., Ruan, Y. J., Rao, M. and Lim, B. (2005) Transcriptome profiling of human and murine ESCs identifies divergent paths required to maintain the stem cell state. Stem cells. **23**, 166-185
6 James, D., Levine, A. J., Besser, D. and Hemmati-Brivanlou, A. (2005) TGFbeta/activin/nodal signaling is necessary for the maintenance of pluripotency in human embryonic stem cells. Development. **132**, 1273-1282
7 Xu, R. H., Sampsell-Barron, T. L., Gu, F., Root, S., Peck, R. M., Pan, G., Yu, J., Antosiewicz-Bourget, J., Tian, S., Stewart, R. and Thomson, J. A. (2008) NANOG is a direct target of TGFbeta/activin-mediated SMAD signaling in human ESCs. Cell stem cell. **3**, 196-206
8 Niwa, H., Burdon, T., Chambers, I. and Smith, A. (1998) Self-renewal of pluripotent embryonic stem cells is mediated via activation of STAT3. Genes Dev. **12**, 2048-2060
9 Ying, Q. L., Nichols, J., Chambers, I. and Smith, A. (2003) BMP induction of Id proteins suppresses differentiation and sustains embryonic stem cell self-renewal in collaboration with STAT3. Cell. **115**, 281-292
10 Romero-Lanman, E. E., Pavlovic, S., Amlani, B., Chin, Y. and Benezra, R. (2012) Id1 maintains embryonic stem cell self-renewal by up-regulation of Nanog and repression of Brachyury expression. Stem cells and development. **21**, 384-393
11 Bandyopadhyay, A., Yadav, P. S. and Prashar, P. (2013) BMP signaling in development and diseases: a pharmacological perspective. Biochemical pharmacology. **85**, 857-864
12 Mishina, Y., Suzuki, A., Ueno, N. and Behringer, R. R. (1995) Bmpr encodes a type I bone morphogenetic protein receptor that is essential for gastrulation during mouse embryogenesis. Genes & Development. **9**, 3027-3037
13 Baron, M. H. and Fraser, S. T. (2005) The specification of early hematopoiesis in the mammal. Current opinion in hematology. **12**, 217-221
14 Zhang, H. and Bradley, A. (1996) Mice deficient for BMP2 are nonviable and have defects in amnion/chorion and cardiac development. Development. **122**, 2977-2986
15 Winnier, G., Blessing, M., Labosky, P. A. and Hogan, B. L. (1995) Bone morphogenetic protein-4 is required for mesoderm formation and patterning in the mouse. Genes Dev. **9**, 2105-2116
16 Beppu, H., Kawabata, M., Hamamoto, T., Chytil, A., Minowa, O., Noda, T. and Miyazono, K. (2000) BMP type II receptor is required for gastrulation and early development of mouse embryos. Developmental biology. **221**, 249-258
17 Johansson, B. M. and Wiles, M. V. (1995) Evidence for involvement of activin A and bone morphogenetic protein 4 in mammalian mesoderm and hematopoietic development. Mol Cell Biol. **15**, 141-151
18 Chadwick, K., Wang, L., Li, L., Menendez, P., Murdoch, B., Rouleau, A. and Bhatia, M. (2003) Cytokines and BMP-4 promote hematopoietic differentiation of human embryonic stem cells. Blood. **102**, 906-915
19 Park, T. S., Zimmerlin, L. and Zambidis, E. T. (2013) Efficient and simultaneous generation of hematopoietic and vascular progenitors from human induced pluripotent stem cells. Cytometry. Part A : the journal of the International Society for Analytical Cytology. **83**, 114-126
20 Lengerke, C. and Daley, G. Q. (2012) Caudal genes in blood development and leukemia. Annals of the New York Academy of Sciences. **1266**, 47-54
21 Lengerke, C., Schmitt, S., Bowman, T. V., Jang, I. H., Maouche-Chretien, L., McKinney-Freeman, S., Davidson, A. J., Hammerschmidt, M., Rentzsch, F., Green, J. B., Zon, L. I. and Daley, G. Q. (2008) BMP and Wnt specify hematopoietic fate by activation of the Cdx-Hox pathway. Cell stem cell. **2**, 72-82
22 Dyer, M. A., Farrington, S. M., Mohn, D., Munday, J. R. and Baron, M. H. (2001) Indian hedgehog activates hematopoiesis and vasculogenesis and can respecify prospective neurectodermal cell fate in the mouse embryo. Development. **128**, 1717-1730

23 Palis, J. and Yoder, M. C. (2001) Yolk-sac hematopoiesis: the first blood cells of mouse and man. Experimental hematology. **29**, 927-936
24 Cumano, A., Dieterlen-Lievre, F. and Godin, I. (1996) Lymphoid potential, probed before circulation in mouse, is restricted to caudal intraembryonic splanchnopleura. Cell. **86**, 907-916
25 Medvinsky, A. and Dzierzak, E. (1996) Definitive hematopoiesis is autonomously initiated by the AGM region. Cell. **86**, 897-906
26 Thambyrajah, R., Mazan, M., Patel, R. and Moignard, V. (2016) GFI1 proteins orchestrate the emergence of haematopoietic stem cells through recruitment of LSD1. **18**, 21-32
27 Bertrand, J. Y., Giroux, S., Golub, R., Klaine, M., Jalil, A., Boucontet, L., Godin, I. and Cumano, A. (2005) Characterization of purified intraembryonic hematopoietic stem cells as a tool to define their site of origin. Proceedings of the National Academy of Sciences of the United States of America. **102**, 134-139
28 Pietila, I. and Vainio, S. (2005) The embryonic aorta-gonad-mesonephros region as a generator of haematopoietic stem cells. APMIS : acta pathologica, microbiologica, et immunologica Scandinavica. **113**, 804-812
29 Medvinsky, A. L. and Dzierzak, E. A. (1998) Development of the definitive hematopoietic hierarchy in the mouse. Developmental and comparative immunology. **22**, 289-301
30 Marshall, C. J., Kinnon, C. and Thrasher, A. J. (2000) Polarized expression of bone morphogenetic protein-4 in the human aorta-gonad-mesonephros region. Blood. **96**, 1591-1593
31 Durand, C., Robin, C., Bollerot, K., Baron, M. H., Ottersbach, K. and Dzierzak, E. (2007) Embryonic stromal clones reveal developmental regulators of definitive hematopoietic stem cells. Proceedings of the National Academy of Sciences of the United States of America. **104**, 20838-20843
32 Drevon, C. and Jaffredo, T. (2014) Cell interactions and cell signaling during hematopoietic development. Experimental cell research. **329**, 200-206
33 Crisan, M., Kartalaei, P. S., Vink, C. S., Yamada-Inagawa, T., Bollerot, K. and van, I. W. (2015) BMP signalling differentially regulates distinct haematopoietic stem cell types. **6**, 8040
34 Crisan, M., Solaimani Kartalaei, P., Neagu, A., Karkanpouna, S., Yamada-Inagawa, T., Purini, C., Vink, C. S., van der Linden, R., van Ijcken, W., Chuva de Sousa Lopes, S. M., Monteiro, R., Mummery, C. and Dzierzak, E. (2016) BMP and Hedgehog Regulate Distinct AGM Hematopoietic Stem Cells Ex Vivo. Stem cell reports. **6**, 383-395
35 Tavian, M., Robin, C., Coulombel, L. and Peault, B. (2001) The human embryo, but not its yolk sac, generates lympho-myeloid stem cells: mapping multipotent hematopoietic cell fate in intraembryonic mesoderm. Immunity. **15**, 487-495
36 Toofan, P., Irvine, D., Hopcroft, L., Copland, M. and Wheadon, H. (2014) The role of the bone morphogenetic proteins in leukaemic stem cell persistence. Biochemical Society transactions. **42**, 809-815
37 Sands, W. A., Copland, M. and Wheadon, H. (2013) Targeting self-renewal pathways in myeloid malignancies. Cell communication and signaling : CCS. **11**, 33
38 Schepers, K., Campbell, T. B. and Passegue, E. (2015) Normal and leukemic stem cell niches: insights and therapeutic opportunities. Cell stem cell. **16**, 254-267
39 Somervaille, T. C., Matheny, C. J., Spencer, G. J., Iwasaki, M., Rinn, J. L., Witten, D. M., Chang, H. Y., Shurtleff, S. A., Downing, J. R. and Cleary, M. L. (2009) Hierarchical maintenance of MLL myeloid leukemia stem cells employs a transcriptional program shared with embryonic rather than adult stem cells. Cell stem cell. **4**, 129-140
40 Massague, J. (2012) TGFbeta signalling in context. Nature reviews. Molecular cell biology. **13**, 616-630
41 Zhang, J., Niu, C., Ye, L., Huang, H., He, X., Tong, W. G., Ross, J., Haug, J., Johnson, T., Feng, J. Q., Harris, S., Wiedemann, L. M., Mishina, Y. and Li, L. (2003) Identification of the haematopoietic stem cell niche and control of the niche size. Nature. **425**, 836-841

42 Soderberg, S. S., Karlsson, G. and Karlsson, S. (2009) Complex and context dependent regulation of hematopoiesis by TGF-beta superfamily signaling. Annals of the New York Academy of Sciences. **1176**, 55-69
43 Larsson, J. and Karlsson, S. (2005) The role of Smad signaling in hematopoiesis. Oncogene. **24**, 5676-5692
44 Bhatia, M., Bonnet, D., Wu, D., Murdoch, B., Wrana, J., Gallacher, L. and Dick, J. E. (1999) Bone morphogenetic proteins regulate the developmental program of human hematopoietic stem cells. The Journal of experimental medicine. **189**, 1139-1148
45 Topic, I., Ikic, M., Ivcevic, S., Kovacic, N., Marusic, A., Kusec, R. and Grcevic, D. (2013) Bone morphogenetic proteins regulate differentiation of human promyelocytic leukemia cells. Leukemia research. **37**, 705-712
46 Laperrousaz, B., Jeanpierre, S., Sagorny, K., Voeltzel, T., Ramas, S., Kaniewski, B., Ffrench, M., Salesse, S., Nicolini, F. E. and Maguer-Satta, V. (2013) Primitive CML cell expansion relies on abnormal levels of BMPs provided by the niche and on BMPRIb overexpression. Blood. **122**, 3767-3777
47 Calvi, L. M., Adams, G. B., Weibrecht, K. W., Weber, J. M., Olson, D. P., Knight, M. C., Martin, R. P., Schipani, E., Divieti, P., Bringhurst, F. R., Milner, L. A., Kronenberg, H. M. and Scadden, D. T. (2003) Osteoblastic cells regulate the haematopoietic stem cell niche. Nature. **425**, 841-846
48 Pilon, N., Oh, K., Sylvestre, J. R., Bouchard, N., Savory, J. and Lohnes, D. (2006) Cdx4 is a direct target of the canonical Wnt pathway. Developmental biology. **289**, 55-63
49 Jamieson, C. H., Ailles, L. E., Dylla, S. J., Muijtjens, M., Jones, C., Zehnder, J. L., Gotlib, J., Li, K., Manz, M. G., Keating, A., Sawyers, C. L. and Weissman, I. L. (2004) Granulocyte-macrophage progenitors as candidate leukemic stem cells in blast-crisis CML. The New England journal of medicine. **351**, 657-667
50 Mikesch, J. H., Steffen, B., Berdel, W. E., Serve, H. and Muller-Tidow, C. (2007) The emerging role of Wnt signaling in the pathogenesis of acute myeloid leukemia. Leukemia. **21**, 1638-1647
51 Charite, J., de Graaff, W., Consten, D., Reijnen, M. J., Korving, J. and Deschamps, J. (1998) Transducing positional information to the Hox genes: critical interaction of cdx gene products with position-sensitive regulatory elements. Development. **125**, 4349-4358
52 Scholl, C., Bansal, D., Dohner, K., Eiwen, K., Huntly, B. J., Lee, B. H., Rucker, F. G., Schlenk, R. F., Bullinger, L., Dohner, H., Gilliland, D. G. and Frohling, S. (2007) The homeobox gene CDX2 is aberrantly expressed in most cases of acute myeloid leukemia and promotes leukemogenesis. The Journal of clinical investigation. **117**, 1037-1048
53 Giampaolo, A., Felli, N., Diverio, D., Morsilli, O., Samoggia, P., Breccia, M., Lo Coco, F., Peschle, C. and Testa, U. (2002) Expression pattern of HOXB6 homeobox gene in myelomonocytic differentiation and acute myeloid leukemia. Leukemia. **16**, 1293-1301
54 Buske, C. and Humphries, R. K. (2000) Homeobox genes in leukemogenesis. International journal of hematology. **71**, 301-308
55 Grier, D. G., Thompson, A., Kwasniewska, A., McGonigle, G. J., Halliday, H. L. and Lappin, T. R. (2005) The pathophysiology of HOX genes and their role in cancer. The Journal of pathology. **205**, 154-171
56 Borrow, J., Shearman, A. M., Stanton, V. P., Jr., Becher, R., Collins, T., Williams, A. J., Dube, I., Katz, F., Kwong, Y. L., Morris, C., Ohyashiki, K., Toyama, K., Rowley, J. and Housman, D. E. (1996) The t(7;11)(p15;p15) translocation in acute myeloid leukaemia fuses the genes for nucleoporin NUP98 and class I homeoprotein HOXA9. Nature genetics. **12**, 159-167
57 Nakamura, T., Largaespada, D. A., Lee, M. P., Johnson, L. A., Ohyashiki, K., Toyama, K., Chen, S. J., Willman, C. L., Chen, I. M., Feinberg, A. P., Jenkins, N. A., Copeland, N. G. and Shaughnessy, J. D., Jr. (1996) Fusion of the nucleoporin gene NUP98 to HOXA9 by the chromosome translocation t(7;11)(p15;p15) in human myeloid leukaemia. Nature genetics. **12**, 154-158
58 Schoch, C., Schnittger, S., Klaus, M., Kern, W., Hiddemann, W. and Haferlach, T. (2003) AML with 11q23/MLL abnormalities as defined by the WHO classification: incidence, partner

chromosomes, FAB subtype, age distribution, and prognostic impact in an unselected series of 1897 cytogenetically analyzed AML cases. Blood. **102**, 2395-2402
59 Camos, M., Esteve, J., Jares, P., Colomer, D., Rozman, M., Villamor, N., Costa, D., Carrio, A., Nomdedeu, J., Montserrat, E. and Campo, E. (2006) Gene expression profiling of acute myeloid leukemia with translocation t(8;16)(p11;p13) and MYST3-CREBBP rearrangement reveals a distinctive signature with a specific pattern of HOX gene expression. Cancer research. **66**, 6947-6954
60 Kim, D. Y., Choi, S. J., Kim, S. H., Chung, H. Y., Yi, S., Kim, D. W., Kim, C. C. and Han, T. H. (2005) Upregulated hoxC4 induces CD14 expression during the differentiation of acute promyelocytic leukemia cells. Leukemia & lymphoma. **46**, 1061-1066
61 Sauvageau, G., Thorsteinsdottir, U., Hough, M. R., Hugo, P., Lawrence, H. J., Largman, C. and Humphries, R. K. (1997) Overexpression of HOXB3 in hematopoietic cells causes defective lymphoid development and progressive myeloproliferation. Immunity. **6**, 13-22
62 Perkins, A., Kongsuwan, K., Visvader, J., Adams, J. M. and Cory, S. (1990) Homeobox gene expression plus autocrine growth factor production elicits myeloid leukemia. Proceedings of the National Academy of Sciences of the United States of America. **87**, 8398-8402
63 Thorsteinsdottir, U., Mamo, A., Kroon, E., Jerome, L., Bijl, J., Lawrence, H. J., Humphries, K. and Sauvageau, G. (2002) Overexpression of the myeloid leukemia-associated Hoxa9 gene in bone marrow cells induces stem cell expansion. Blood. **99**, 121-129
64 Antonchuk, J., Sauvageau, G. and Humphries, R. K. (2002) HOXB4-induced expansion of adult hematopoietic stem cells ex vivo. Cell. **109**, 39-45
65 Strathdee, G., Holyoake, T. L., Sim, A., Parker, A., Oscier, D. G., Melo, J. V., Meyer, S., Eden, T., Dickinson, A. M., Mountford, J. C., Jorgensen, H. G., Soutar, R. and Brown, R. (2007) Inactivation of HOXA genes by hypermethylation in myeloid and lymphoid malignancy is frequent and associated with poor prognosis. Clinical cancer research : an official journal of the American Association for Cancer Research. **13**, 5048-5055

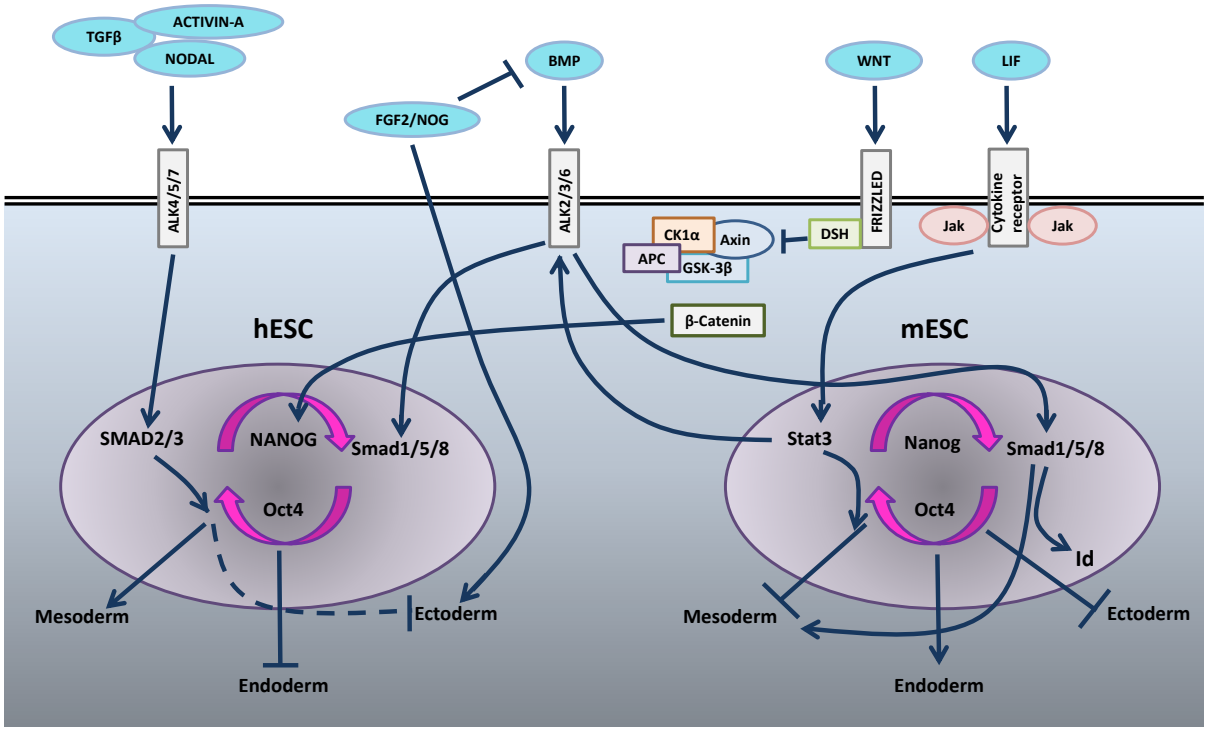

Figure 1. Maintenance of pluripotency in mouse and human stem cells. In human ESCs (hESCs) inhibition of BMP4 via the action of fibroblast growth factor 2 (FGF2) and noggin (NOG), phosphorylation of SMAD2/3, and activation of the PI3K/AKTpathway and stabilisation of β-catenin have been suggested to be crucial for the maintenance of pluripotency. Downregulation of NANOG and OCT4 results in differentiation of hESCs to extraembryonic lineages (ectoderm and endoderm), indicating that each of these transcription factors is important for the maintenance of pluripotency. TGFβ/Nodal/Activin signalling pathways inhibit differentiation to ectoderm and promote pluripotency in hESCs. In murine embryonic stem cells (mESCs) Bone morphogenetic protein 4 (BMP4) and leukaemia inhibitory factor (LIF) signalling are required to maintain the pluripotency under serum-free culture conditions. BMP4 signalling activates the expression of inhibitor of DNA binding (Id) genes through the phosphorylation of downstream smads 1/5/8. This inhibits mESC differentiation to neuroectoderm. LIF signalling results in activation of transcription factors (Jak/Stat3) and suppression of differentiation to endodermal and mesodermal lineages. Nanog and Oct4 action is required for both the self-renewal of mESCs and maintenance of pluripotency since their down regulation results in differentiation towards extraembryonic lineages and loss of self-renewal (pink arrows: interaction between the components, blue arrows: activation, blunt end lines: inhibition, dotted lines: indirect effect).

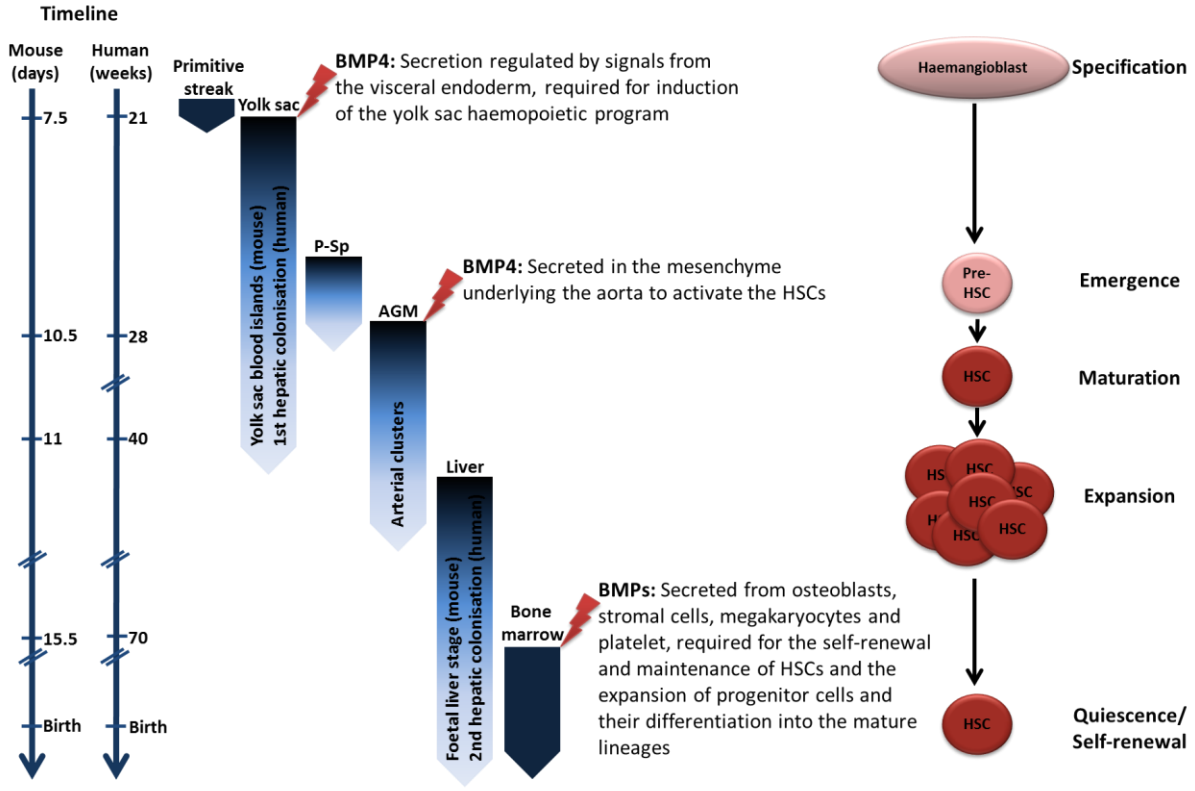

Figure 2. Haemopoietic development timeline in mouse and human. The site of primary haemopoiesis in the embryo is transient which means that it changes during development. This process involves several anatomical sites (the yolk sac, the aorta-gonad-mesonephros region, the placenta and the foetal liver), after which HSCs colonize the bone marrow. Mesoderm is formed during gastrulation. Commitment of posterior mesoderm to haemangioblasts occurs at a very early stage in the primitive streak. Primitive haemopoiesis and vasculogenesis become specified from the haemangioblasts where commitment of mesodermal progenitors to the haemopoietic and endothelial lineages begins. This is then followed by development of yolk sac blood islands. Definitive haemopoiesis is initiated by the emergence of HSCs in the AGM region as well as in large arteries and placenta. HSCs then migrate and expand in the foetal liver followed by the bone marrow of the late gestation foetus. Pre-HSCs undergo a maturation process that allows them to engraft, survive and self-renew in future haemopoietic niches. Subsequently, foetal HSCs expand rapidly, after which a steady state is established in which HSCs reside in a relatively quiescent state in the bone marrow after birth (time line on the left of the diagram correlates with the schematic presentation on the right of the diagram. Block colour arrows illustrate the haemopoietic sites being consistently active whereas colour gradient arrows show the site loses its role during haemopoietic ontogeny).

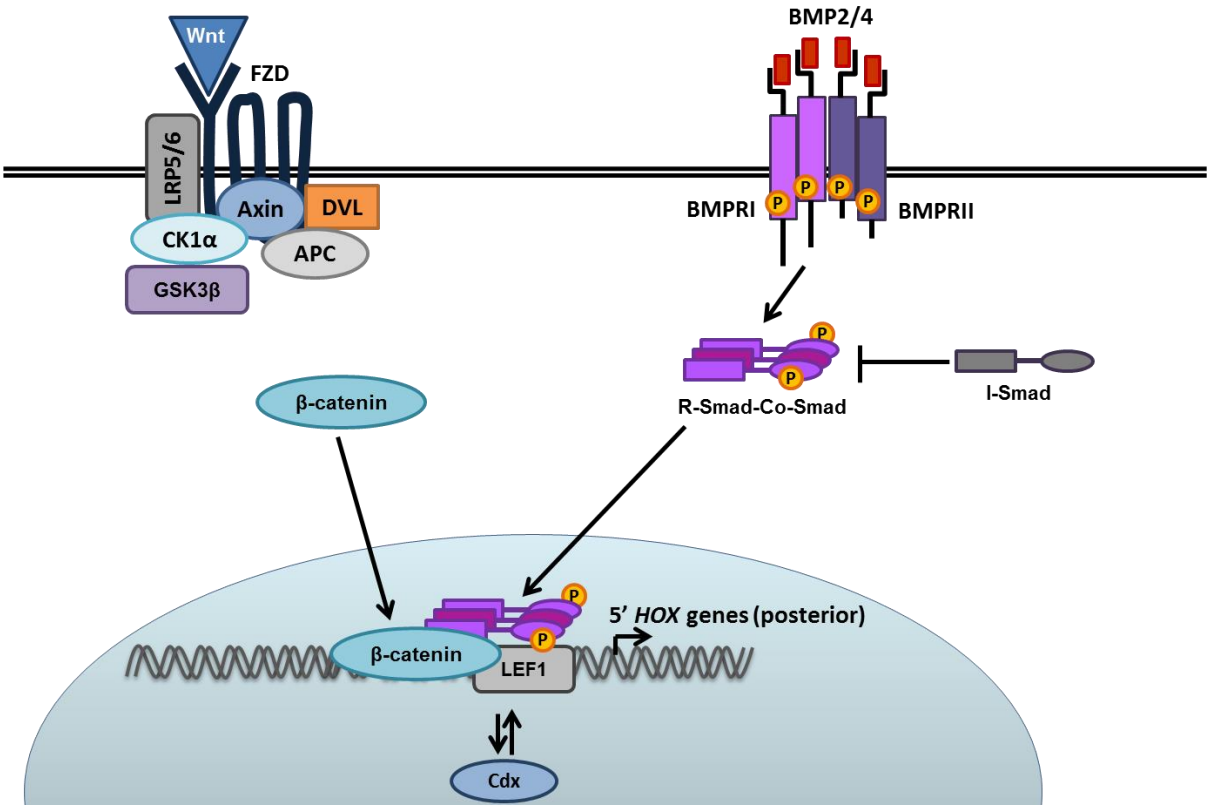

Figure 3. Main morphogenic signalling pathways activating Hox genes. Posterior HOX genes are predominantly activated by canonical Wnt and non-canonical BMP pathways. In the presence of Wnt ligand, Wnt binds to frizzled (FZD) receptor and LRP5/6 co-receptor and activates dishevelled (DVL), leading to the inhibition of APC/Axin/GSK3β-mediated β-catenin degradation. Phosphorylated SMAD1/5/8 through non-canonical BMP pathway form a complex with stabilised β-catenin and LEF1 at the promoter region of posterior HOX genes and regulate their expression with the aid of Cdx family members.