**Evolutionarily Conserved Signaling Pathways: Acting in the Shadows of Acute Myelogenous Leukemia’s Genetic Diversity**

Florian H. Heidel, Patricia Arreba-Tutusaus, Scott A. Armstrong, and Thomas Fischer

**Abstract**

Acute myelogenous leukemia stem cells (AML–LSC) give rise to the leukemic bulk population and maintain disease. Relapse can arise from residual LSCs that have distinct sensitivity and dependencies when compared with the AML bulk. AML–LSCs are driven by genetic and epigenomic changes, and these alterations influence prognosis and clonal selection. Therapies targeting these molecular aberrations have been developed and show promising responses in advanced clinical trials; however, so far success with LSCs has been limited. Besides the genetic diversity, AML–LSCs are critically influenced by the microenvironment, and a third crucial aspect has recently come to the fore: A group of evolutionarily conserved signaling pathways such as canonical Wnt signaling, Notch signaling, or the Hedgehog pathway can be essential for maintenance of AML–LSC but may be redundant for normal hematopoietic stem cells. In addition, early reports suggest also regulators of cell polarity may also influence hematopoietic stem cells and AML biology. Interactions between these pathways have been investigated recently and suggest a network of signaling pathways involved in regulation of self-renewal and response to oncogenic stress. Here, we review how recent discoveries on regulation of AML–LSC-relevant evolutionarily conserved pathways may open opportunities for novel treatment approaches eradicating residual disease.

**Disclosure of Potential Conflicts of Interest**

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**Learning Objectives**

Upon completion of this activity, the participant should be able to understand the roles of Notch–, Wnt–, and Hedgehog–signaling in the development and maintenance of AML stem cells and their potential applications in the new generation of targeted therapeutics for AML.

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**Introduction**

Development of next-generation sequencing (NGS) techniques has facilitated detailed analysis of genetic heterogeneity in acute myelogenous leukemia (AML) and enabled detection of novel mutations with distinct functional properties (1–3). Genetic diversity, however, is not the only determinant of relapse, drug resistance, and aggressiveness of leukemia biology. Multiple determinants shape hierarchical organization of the leukemic bulk, where a substantial population of leukemia stem cells (LSC) maintains disease.

Aspects that influence leukemia stem cell biology and self-renewal include (i) genetic and epigenomic alterations, (ii) alterations of the bone marrow niche, and (iii) nongenetic determinants, such as evolutionarily conserved signaling pathways (ECSP; Fig. 1).

Genetic alterations are not homogeneously present within a single individual. AML presents rather as a heterogeneous mixture of genetically distinct subclones arising through branching
evolution (4, 5), and minor resistant subclones may emerge as the origin of relapse after discontinuation of chemotherapy.

Location of hematopoietic stem cells (HSC) and LSCs in the bone marrow niche is able to modify its function by cross-talk of the stem cell with the stromal cells forming the niche.

Adhesion molecules, membrane receptors, and secretion of chemokines influence proliferation and drug resistance irrespective of the underlying genetic diversity. Microenvironmental changes may even drive malignant transformation of HSCs within the niche and lead to activation of signaling pathways as well as secondary genetic alterations. Interaction with the niche may also influence the third determinant of AML heterogeneity: ECSPs.

Although some signaling pathways are frequently mutated in AML and act as drivers of malignant transformation for HSCs, progenitor cells, and the bulk population, other ECSPs are neither frequently mutated nor necessarily relevant for the AML bulk. These ECSPs modulate self-renewal capacity of stem cells. Self-renewal is considered to be the integral property of both HSC and LSC, and its deregulation is known to affect development and maintenance of AML–LSC (6). Recent data indicate that ECSPs play an important role in stem cell development, with differential requirement of these signaling pathways in maintenance of stem cell hierarchies. Importantly, some ECSPs seem dispensable for maintenance of normal adult HSC, whereas LSC seems to retain dependency on specific signaling nodes (6, 7). Another important aspect is based on the underlying genetic diversity: Expression of specific oncogenes such as MLL fusions (fusions affecting the mixed-lineage-leukemia gene on chromosome 11q23) may create new dependencies on specific ECSPs in LSCs. This concept of acquired vulnerability may create novel therapeutic target structures in AML. Targeting dependency on ECSPs that are otherwise dispensable for maintenance of adult hematopoiesis may offer a therapeutic window to eradicate minimal residual disease (MRD).

Although the role of various ECSPs has been described in ontogenesis and stem cell development, this review focuses on a group of ECSPs that have been described in the context of disease development, biology, and prognosis of AML. They represent a unique class of AML-relevant signaling pathways acting in the shadows of genetic diversity and cell-niche interaction.

Notch signaling

Notch receptors are an evolutionarily conserved family of transmembrane proteins that are expressed and active in normal HSC (8, 9). The Notch pathway is highly regulated and requires a specific cell–cell interaction between Notch and its ligand (Fig. 2; refs. 10, 11). In lymphoid malignancies, an oncogenic role of Notch was anticipated, and several groups provided evidence that activating mutations of Notch contributed to development of T-cell acute lymphoblastic leukemia (T-ALL; ref. 12) or chronic lymphocytic leukemia (13).

Notch is essential for the development of HSCs during embryonic blood formation (Table 1; ref. 14). There are conflicting data on the role of Notch signaling in the function of adult HSCs and myeloid differentiation. These differences may be explained by the experimental approach and the extent of Notch modulation. Genetic deletion of Notch receptors or modulation of ligands and complex members does not impair adult HSC function (15, 16). However, abrogation of Notch signaling by conditional deletion of Nicastin—a crucial component of the g-secretase complex—led to transformation of adult HSCs into a preleukemic state (17). Cross-talk between Wnt and Notch signaling pathways as described in colon cancer models may also predict potential influence of Notch signaling on Wnt-dependent AML (Fig. 2; ref. 18). For vivo investigation of AML cell lines and primary patient blasts revealed downregulation of Notch1 expression to be associated with a decrease in PU.1-mediated differentiation capacity. This indicated for the first time a crucial role in maintenance of an immature state (19). Until most recently, the relevance of Notch signaling in AML, especially in self-renewal of AML–LSC, remained elusive. Recently, publications demonstrated a role for Notch expression in AML and activation of Notch signaling as a potential therapeutic opportunity (17, 20): Notch receptors have been shown to be expressed in AML, however, expression of Notch downstream targets was extremely low, indicating silencing of Notch signaling both in both human and murine AML, which may be influenced by underlying genetic alterations. In contrast, activation of Notch signaling either by activating mutations in vivo or ligand stimulation in vitro led to significant induction of differentiation, apoptosis, and cycle arrest in AML–LSC (17). Inactivation of Notch signaling has been described to initiate a chronic myelomonocytic leukemia (CMMIL)–like phenotype in vivo (21) comparable with the phenotype achieved by deletion of the hydroxymethylation pathway regulator Tet2. Combination of Notch inactivation with Tet2 deletion led to overt AML, indicating cooperation in AML development. This suggests a role of reduced Notch target gene expression in early development of AML generating a preleukemic state.

As outlined before, cell intrinsic mutations that activate Notch signaling have been found in lymphoid neoplasia, while not being reported in myeloid malignancies. Functional investigation in experimental mouse models confirmed that activation of Notch signaling is thought to contribute to acute lymphoblastic leukemia (ALL), whereas abrogation of Notch signaling may contribute to myelodysplasia (MDS) or eventually AML. Most recently, activation of Notch through the bone marrow...
Jagged expression is activated in osteoblasts in AML and MDS.

CTNNB1 is stabilized/activated in AML and myeloid LSC.

Notch activity is reduced in myeloid malignancy.

CTNNB1 is activated in AML and MDS.

Target gene transcription
Activated through double proteolytic cleavage of the receptor. First, the receptor becomes extracellularly modified by delta/Jagged ligands expressed on neighboring cells bind to the extracellular domains of Notch receptor. In mammalians, four receptor isoforms (Notch1-4) exist with five canonical notch ligands being classified into Jagged and delta-like families (Jag1, Jad2, Dll1, Dll3, and Dll4; ref. 9). Upon ligand binding, the pathway is activated through double proteolytic cleavage of the receptor. First, the receptor becomes extracellularly modified (S2 site) by an A-Dissociase (ADAM) Metallopeptase (ADAM), and subsequently, the notch intracellular domain (NICD) is released by a γ-secretase/presenilin complex. The NICD travels from the cytoplasm to the nucleus, forming an active complex with the DNA-binding transcription factor CSL/RBP-Jk (9). The complex is stabilized by the coactivator mastermind-like (MAML1–3), among others, switching on the transcription of target genes, such as Hes family genes, c-Myc, cyclins D1 and D3, or Notch 1 and 3 (10). C. In the absence of Wnt ligand (inactive), β-catenin is recruited by a multifactor complex formed by glycogen synthase kinase-3β (GSK-3β) and reconstitution of HSC. Modest activation of canonical Wnt signaling through (haplo-insufficiency of the Apcmin-mutant) led to competitive advantage of HSC; however, these cells were exhausted after the second round of transplantation (31). Pronounced activation of Wnt signaling (Apic inactivation) impairs HSC self-renewal and differentiation (29) through unlatched cell-cycle activity of HSCs followed by loss of their function and apoptosis (32).

Canonical Wnt signaling

Wnt signaling plays a critical role in embryonic and hematopoietic development. Wnt ligands are secreted glycoproteins, which can be released or presented on the cell surface and induce different pathways. The canonical Wnt pathway has been studied in great detail with β-catenin (Ctnnb1) being the central player of the signaling cascade (Fig. 2A and B).

During development, canonical Wnt signaling plays an important role in generation of normal HSPCs (Table 1; refs. 26, 27). Ctnnb1 deletion in early HSC development does not affect HSC establishment, but HSCs are impaired in long-term growth and maintenance following transplantation (27). In contrast, conditional inactivation of both β- and γ-catenin in adult, steady-state HSC does not cause significant perturbation of hematopoiesis (28). This indicates differential requirement for canonical Wnt signaling in development versus maintenance of adult HSCs. Activation of Ctnnb1 has variable effects on HSCs depending on the magnitude of activation (29). These dose-dependent effects may explain the variability of Ctnnb1 requirement in different models (30). Mild activation of Wnt signaling modified HSC function toward self-renewal, resulting in improved maintenance and reconstitution of HSC. Modest activation of canonical Wnt signaling through (haplo-insufficiency of the Apcmin-mutant) led to competitive advantage of HSC; however, these cells were exhausted after the second round of transplantation (31). Pronounced activation of Wnt signaling (Apic inactivation) impairs HSC self-renewal and differentiation (29) through unlatched cell-cycle activity of HSCs followed by loss of their function and apoptosis (32). Likewise, significant activation of Ctnnb1 through inactivation of its inhibitor GSK3β leads to expansion of the HSC pool, followed by stem cell exhaustion and bone marrow failure (33, 34).

Wnt signaling is activated in both AML–LSC (35) and myeloid blast crisis of chronic myelogenous leukemia (CML; ref. 36). In primary AML patient samples, immunohistochemical expression and constitutive activation of canonical Wnt pathway member β-catenin were also detectable in the bulk AML population (37). High expression of Ctnnb1 has been reported to correlate with poor prognosis, and γ-catenin seems to stabilize the nuclear "active" version of β-catenin in AML cells (38, 39). Of note, abundance of Ctnnb1 in these subentities can be considered to be a direct result of either transcriptional or posttranslational activation through the respective genetic alteration itself. Constitutive activation of oncogenic tyrosine kinases can stabilize αngious transformation in another cell type or lineage.

**Table 1. Requirement of evolutionarily conserved pathways in normal HSC and AML–LSC.**

<table>
<thead>
<tr>
<th>Pathway</th>
<th>HSC development</th>
<th>Adult HSC maintenance</th>
<th>AML–LSC development</th>
<th>AML–LSC maintenance</th>
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<tr>
<td>Notch 1</td>
<td>(+)</td>
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<td>Wnt</td>
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**NOTE:** +, required; (+), context-dependent requirement; −, not required.

The microenvironment has been shown to promote leukemogenesis in AML. Interestingly, Notch activity is triggered by constitutive activation of the Wnt signaling molecule β-catenin (Ctnnb1) caused by an activating mutation of Ctnnb1 in bone marrow osteoblasts (22). This constitutive activation of Ctnnb1 leads to overexpression of the Notch-ligand jagged 1 and results in exogenous activation of Notch signaling in hematopoietic stem/progenitor cell (HSPC). This activating mutation of Ctnnb1 has been reported in up to 38% of patients with MDS or AML. This adds another layer of complexity to the dual role of Notch in myeloid malignancies.

Inhibitors of Notch signaling have already been developed and tested in T-cell leukemia, in which gain-of-function mutations are frequent. γ-secretase inhibitors (GSI) MK-0752 and PF03084014 have, therefore, been tested in T-ALL whereas others are currently being investigated in early-phase clinical trials for solid tumors (Table 2; ref. 23). Monoclonal Notch-receptor antibodies (24), soluble receptor decoys, or RNAi are still under development in preclinical stages.

Described as an oncogene in ALL, Notch 1 seems to exert tumor-suppressor activity in AML. This dual role is highly cell context dependent and may be influenced by cell-type–specific genetic alterations. The potential suitability of Notch activation to serve as a target in AML–LSC now adds another layer of complexity to the development of Notch-targeted therapies. Activating agents of the Notch pathway such as Notch1 agonistic antibodies have been successfully tested in animal models of different biologic context (25). Others, such as activating Notch ligand or small-molecule agonists, could be developed for targeting MRD in subtypes of AML (17). However, given the dual role of Notch signaling in hematopoiesis (Table 1), it is difficult to predict the effects of novel therapeutic approaches. Thus, while targeting the malignant clone in one lineage, one might cause (pre-) malignant transformation in another cell type or lineage.

**Figure 2.**

Canonical Notch (A) and Wnt signaling (B, simplified view). A, inactive Notch receptor is known to promote ubiquitylation and proteasomal degradation of phosphorylated β-catenin (the central signaling node of the Notch Wnt pathway), indicating interaction between these evolutionarily conserved pathways. B, delta/Jagged ligands expressed on neighboring cells bind to the extracellular domains of Notch receptors. In mammals, four receptor isoforms (Notch1-4) exist with five canonical notch ligands being classified into Jagged and delta-like families (Jag1, Jad2, Dll1, Dll3, and Dll4; ref. 9). Upon ligand binding, the pathway is activated through double proteolytic cleavage of the receptor. First, the receptor becomes extracellularly modified (S2 site) by an A-Dissociase (ADAM) Metallopeptase (ADAM), and subsequently, the notch intracellular domain (NICD) is released by a γ-secretase/presenilin complex. The NICD travels from the cytoplasm to the nucleus, forming an active complex with the DNA-binding transcription factor CSL/RBP-Jk (9). The complex is stabilized by the coactivator mastermind-like (MAML1–3), among others, switching on the transcription of target genes, such as Hes family genes, c-Myc, cyclins D1 and D3, or Notch 1 and 3 (10). C. In the absence of Wnt ligand (inactive), β-catenin is recruited by a multifactor complex formed by glycogen synthase kinase-3β (GSK-3β; ref. 65), casein kinase 1 (CK1), adenomatous polyposis coli (APC) a tumor-suppressor protein, and the scaffold protein Axin (66). Axin promotes GSK-3β-dependent phosphorylation of β-catenin for the ubiquitin–proteasome pathway, maintaining low levels of the protein. D, upon Wnt binding to the frizzled (FZD) and LRP5/6 coreceptor complex (active), β-catenin phosphorylation is blocked. This leads to cytoplasmatic accumulation of unphosphorylated β-catenin that can shuttle to the nucleus. Nuclear β-catenin binds to T-cell factor (TCF)/lymphoid enhancer factor (LEF) transcription factors to promote expression of target genes, such as c-myc, cyclin D1, or c-jun (67). Recently, detailed investigation of canonical Wnt signaling revealed a novel mechanism of Ctnnb1 stabilization. Here, β-catenin requires the association of Axin with the phosphorylated LRPS-receptor complex, followed by a destruction of the represcer complex and an accumulation of newly translated β-catenin in the cytosol (68). Prostaglandin (PGE2) signaling is known to modulate Wnt pathway activity through PKA/cAMP-mediated signaling.
activate Wnt signaling in a cell-intrinsic manner. Heidel et al. (Table 1; refs. 27, 40) identified that certain subtypes of AML with Ctnnb1 rearrangements revealed that self-renewal capacity of LSC is mediated—at least in part—by Ctnnb1 (41, 42). Constitutively expressed Ctnnb1 enabled progenitor cells to form leukemia with a similar efficacy as the corresponding stem cell controls (41). On the other hand, genetic deletion of Ctnnb1 led to reduction of LSC and thus to decreased leukemia formation. Interestingly, these effects could be mimicked using pharmacologic treatment, confirming the importance of Ctnnb1 for maintenance of AML–LSC. Interference of prostaglandin signaling has been shown to target Wnt/β-catenin axis in HSC (43, 44), and abrogation of Ctnnb1 by the COX inhibitor indomethacin led to a 100-fold decrease in AML–LSC in secondary recipients (41). Moreover, COX inhibition of fully developed MLL-AF9–induced leukemia led to reduction of Ctnnb1 and of LSC frequency. These data indicate that certain subtypes of AML–LSC retain dependency on canonical Wnt signaling and suggest that self-renewal pathways can be selective therapeutic targets for LSC (45). In contrast, activation or accumulation of Ctnnb1 has been shown to mediate oncogenic potential (31, 36) in human and murine leukemia models and modest activation creates a preleukemic state in vivo (31).

Wnt–Notch interaction has been described in regard to the bone marrow stroma. As outlined above, mutations of Ctnnb1 have been found in bone marrow osteoblasts, resulting in overexpression of Notch ligands and consecutive activation of Notch signaling in HSPCs (22). Therapeutically, development of Ctnnb1-targeting drugs remains a challenge. Several relevant pathway members have been used as target structures with varying success. Compounds such as XAV939 have been discovered recently and stabilize Axin by inhibition of the enzymes Tankyrase 1 and 2, thus promoting degradation of Ctnnb1. Tankyras may serve as a bona fide target in colon cancer (46); however, their role in HSC appears more complicated. Antibodies directed against LRP receptors may influence the niche–cell interaction rather than cell intrinsic activation of Wnt signaling in AML. Recently, inhibitors of Ctnnb1 itself have been presented in preclinical studies of leukemia cells lines. Inhibitors of canonical Wnt signaling have not yet reached clinical trials for AML.

Hedgehog

The Hedgehog (Hh) signaling pathway is highly conserved in vertebrates (47), and three Hedgehog isoforms have been described in hematopoiesis: Sonic- (Shh), Desert- (Dhh), and Indian-Hedgehog (Ihh). The receptor, Patched (Ptc), acts as a negative modulator. In absence of Hedgehog, the negative modulator Patched represses the signal-transducer Smothened (Smo; Fig. 3A and B; refs. 47, 48), which enables activation of β-catenin loss of function. However, Hedgehog signaling has been shown to be dispensable for maintenance of normal adult HSCs in conditional knockout mouse models (Table 1; refs. 51, 52).

Ablation of Hedgehog signaling has been found in LSC. However, no convincing data are published on gene-expression changes of the Hedgehog pathway and implications of expression levels on prognosis or disease biology in AML with the exception of a recent report on Hedgehog activity in acute promyelocytic leukemia (53). Experimental data on the relevance and influence of Hedgehog signaling on AML–LSC are more limited than for Notch or canonical Wnt signaling. In regard to its role in myeloid neoplasia, most data have been published on the role of Hedgehog signaling inhibitors in CML (49, 50). Here, genetic inactivation of Smothened led to decreased penetrance and increased latency of CML (50). Similar effects could be observed using the Hedgehog inhibitor cyclopamine. In contrast, Hedgehog was described to be dispensable in maintenance of AML–LSC. Genetic inactivation of Smothened in MLL-AF9–transformed LSC did not affect AML development in primary recipient mice (52).

Several small-molecule inhibitors of Hedgehog signaling have been developed for solid cancer (Table 2). Although the preclinical experimental data are less convincing in comparison with other ESCPs, Hedgehog inhibitors are being tested in a variety of myeloid malignancies. Currently, clinical trials are under way to investigate Hedgehog signaling inhibitors in AML: LDE225 and PF04449913 are evaluated in international multicenter phase II trials either as monotherapy or combination with chemotherapy (23). Of note, the LDE225 trial stopped recruitment in early 2014 due to lack of efficacy, highlighting the limited activity of Smothened inhibition on the rapidly proliferating leukemic bulk.

Polarity regulators and cell fate determinants

Loss of cell polarity can influence tumor development by altering cell–cell matrix interactions. Moreover, changes in cell
Decreased expression of LLGL1 in AML is associated with dismal prognosis. LLGL2 mutation is detected in AML.

High expression of MSI2 in AML is associated with dismal prognosis.
polarity are essential for regulation of symmetric versus asymmetric cell division in HSC and LSC. Only few of these proteins have been investigated in detail. Evidence for a role of Musashi-2 (Msi-2) not only in regulation of HSC but also AML–LSC has been provided recently (Fig. 3D). Although data are still limited to a few reports, recent findings suggest involvement of Scribble polarity complex proteins (Llgl1 and 2) in regulation of HSC polarity and potential implications in AML biology (Fig. 3C).

Msi-2 is a known regulator of the HSC pool and of HSC activity (54–56). Loss of Msi-2 was associated with reduction in short-term-HSCs (ST-HSC), impaired proliferation capacity, and competitive disadvantage. RNAi-based knockdown of short-term-HSCs (ST-HSC), impaired proliferation capacity, and competitive disadvantage. RNAi-based knockdown of Msi-2 also impaired long-term HSC (LT-HSC) function. Long-term-HSCs are maintained through (a)symmetric cell division and this process is regulated by Msi-2 (57). Using a conditional knockout mouse model, genetic inactivation of Msi-2 severely impaired LT-HSC function as Msi-2–/– HSC become insensitive to TGFβ-mediated LT-HSC expansion (57). In AML, protein expression of Msi-2 was associated with unfavorable prognosis in AML independently of other known risk factors (58). Consistently, gene expression of Msi2 is correlated with dismal overall survival in AML (59). Moreover, Msi-2 has been investigated recently in leukemia mouse models (56, 60). Msi-2 inactivation was shown to be associated with a reduction in both symmetric division (56) and progression of CML (60). Increased expression of Msi2 was associated with aggressive disease and an immature phenotype of human AML and CML (56, 60).

Recently, mutation of Lethal-giant-larvae 2 (Llgl2) has been described as an early mutational event in progression from severe congenital neutropenia to AML (61). Decreased expression of Lgl1, its close homolog in mammals, could be found in AML–LSC of different origin (L-MPP/L-GMP), when compared with their normal counterparts (MPP/GMP; refs. 62, 63). Reduced Lgl1 expression was associated with significantly decreased overall survival in two independent patient cohorts treated for AML (63). However, it cannot be ruled out that these changes may be secondary due to genetic alterations in AML. Genetic inactivation of Lgl1 led to expansion of the HSC pool, suggesting a potential role of decreased expression in disease development. Of note, mouse models investigating the role of Lgl1 in the background of B- and T-cell leukemia did not generate any evidence for tumor-suppressor activity in the genetic models applied (64). These data suggest that the role of Lgl1 in the hematopoietic lineage might be restricted to specific cooperating mutations and a limited number of cellular contexts such as the HSC compartment.

Most recently, a switch from canonical to noncanonical Wnt signaling has been reported in aging of HSC, resulting in loss of cell polarity and skewing of lineage commitment (65). This aging-related process may also influence susceptibility of HSC for malignant transformation.

Summary and Outlook
Factors that influence AML LSCs include (i) genetic/epigenetic variability, (ii) contributions of the bone marrow niche, and (iii) ECSPs that contribute to LSC maintenance. ECSPs can be modulated by underlying genetic as well as the bone marrow microenvironment. Of note, this review focused on detailed investigation of these pathways in various mouse models. However, human HSC/LSC may display a distinct dependency on these pathways and not all findings described may be transferable to human disease. These pathways may offer a novel target for future therapeutic interventions, focusing on eradication of LSC. Therefore, a window needs to be established for therapeutic intervention. Although a distinct requirement for adult HSC compared with LSC may facilitate therapeutic use in regard to hematopoietic toxicity, the requirement of evolutionarily conserved pathways for other somatic tissues needs to be considered. In AML, clinical phase I/II trials targeting prominent ESCP-related molecules have been recently initiated and results are eagerly awaited.

Besides their relevance in maintenance of LSC, these pathways offer therapeutic potential in regard to influencing regenerative potential of normal adult HSC. This is of major interest in the setting of allogeneic stem cell transplantation, which is strongly dependent on efficient homing and engraftment of HSC.

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Figure 3.
Hedgehog signaling pathway and polarity regulators interacting with ESCPs (simplified view). A, the integral part of the Hedgehog signaling pathway is the transmembrane proteins “patched” (Ptc) and “smoothened” (Smo). They are located on the plasma membrane. B, once Hedgehog binds to Patched, Smoothened inhibition is interrupted, allowing its translocation to the membrane and activation by phosphorylation (T). Downstream, Smoothened targets and stabilizes ubiquitin-ligase Itch modulating a number of factors members of the Gli family, which translocate to the nucleus. Gilt and Gili, as activators, switch on the transcription of target genes important in proliferation and survival as well as Gilt and Pthc1/2. On the other hand, Gilt and Gili act as repressive transcription factors, especially when Patched is active and Smoothened is inactive, and therefore not able to repress their degradation. SuFu prevents the active form of Gilt from transactivating Hedgehog-responsive genes. Bona fide Hedgehog target genes include Gilt, Gili, Ptc, and regulators of cell proliferation and survival. C and D, several cell fate determinants and polarity regulators have been described to interact with ECSPs such as canonical Wnt signaling, Hedgehog, or Notch. However, few of these interactions have been confirmed so far in hematopoietic cells. Lgl1 (a member of the “Scribble complex”) interacts with the “Par complex” (Par3, Par6, and aPKCs), parts of which interact closely with canonical Wnt signaling (downstream of Fzd and Strab receptors). C, the Par complex is regulated downstream of G-protein–coupled receptors (GPCR) and CDC42. Moreover, interactions between Lgl1 and the cytoskeleton (microtubules) have been reported. D, Msi-2 is a known interaction partner of Numb, a major determinant of binary cell fates (69, 70). However, the role of the Msi-2–Numb axis in regulation of HSC polarity has been controversially discussed (56). Numb has been described to influence ECSPs (Notch and Hedgehog) through ubiquitinylation and protosomal degradation (69, 71). The E3 ubiquitin–ligase Itch modifies ubiquitinylation of activated Notch and the Hedgehog–intermediate Gli downstream of Numb. Itch negatively regulates the development and function of HSCs (77). In a straight knockout mouse model, genetic inactivation of Itch resulted in enhanced numbers and competitive advantage of HSCs. This gain-of-function can be partially explained by accumulation of activated Notch in Itch-deficient HSC.

Figure 3.


Evolutionarily Conserved Signaling Pathways: Acting in the Shadows of Acute Myelogenous Leukemia's Genetic Diversity


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