Chapter 2

Function of the Stem Cell Transcription Factor SALL4 in Hematopoiesis

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Additional information is available at the end of the chapter

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Abstract

SALL4 is a zinc finger DNA-binding protein that has been well characterized in development and in embryonic stem cell (ESC) maintenance. Notably, SALL4 may be one of the few genes that are also involved in tissue stem cells in adults, and SALL4 protein expression has been correlated with the presence of stem and progenitor cell populations in various organ systems and also in human cancers. In normal hematopoiesis, SALL4 expression is restricted to the rare hematopoietic stem/progenitor cell (HSC/HPC) fractions but is rapidly silenced following lineage differentiation. In hematopoietic malignancies, however, SALL4 is persistently expressed and its expression levels are linked with deteriorated disease status. Furthermore, SALL4 activation participates in the pathogenesis of tumor initiation and disease progression. This chapter summarizes recent advances in our knowledge of SALL4 biology with a focus on its regulatory functions in normal and leukemic hematopoiesis. A better understanding of SALL4's biologic functions and mechanisms is needed to facilitate the development of advanced therapies in future.

Keywords: pluripotency, leukemogenesis, hematopoietic stem/progenitor cell, MLL-rearrangement, epigenetic, histone methylation, DNA methylation, differentiation, zinc finger domain

1. Introduction

SALL4 is one of four human homologs (*SALL-1*, *-2*, *-3*, *-4*) of the Drosophila region-specific gene *Spalt* (*sal*). In Drosophila, *sal* is a homeotic gene essential for development of posterior head and anterior tail segments. As a DNA-binding transcription factor, the SALL4 protein is characterized by multiple Cys2His2 zinc finger (C2H2-ZF) domain distributed over the entire

protein [1–3]. In mammals, the expression of SALL4 has been primarily detected in ESCs and in adult tissue "stem-like" cells, where it mainly activates pluripotency and/or multipotency genes and suppresses differentiation-related genes, thereby modulating the cell "stemness" in development and in tissue generation [4–8]. In humans, heterozygous SALL4 mutation has been linked to Okihiro syndrome, Holt-Oram syndrome, acro-renal-ocular syndrome, and IVIC syndrome, all characterized by multiple organ malformations [9–11]. While normally downregulated or no longer expressed in fully differentiated somatic cells, abnormal reactivation of SALL4 in adult cells may lead to malignancy. To date, aberrant SALL4 expression has been detected in over 10 types of human solid tumors and in several common types of leukemias, and SALL4 has been considered a useful biomarker for these diseases [7, 8, 12, 13]. In addition, studies suggest that SALL4 may be a therapeutic target in treating human leukemias [12, 13]. For these reasons, it will be important to understand how SALL4, a critical pluripotency factor, exerts its effects in different cell contexts, and how we can effectively translate our knowledge gains into treatment breakthroughs in future.

2. SALL4 roles in stem cells and development

2.1. The roles of SALL4 in ESC property maintenance and embryonic development

SALL4 has been one of the most studied transcriptional regulators in ESC self-renewal and pluripotency maintenance. It has been reported that in human ESCs, a well-controlled SALL4/OCT4 transcription regulatory loop balances proper expression dosage of SALL4 and OCT4; and reduction of SALL4, like OCT4, results in re-specification of ESCs to the trophoblast lineage [14–17]. In mouse ESC studies, chromatin immunoprecipitation coupled to microarray hybridization (ChIP-on-chip) revealed that SALL4 binds to about twice as many gene promoters as NANOG and binds about four times more genes than OCT4; and the three factors were found to form heteromeric protein complex in regulating stem cell pluripotency. Further, SALL4 binds many genes that are regulated by chromatin-based epigenetic events mediated by cohesin complex, polycomb-repressive complexes 1 and 2 (PRC1 and PRC2), and bivalent domains [18, 19]. Thus, SALL4 plays a diverse role in regulating stem cell pluripotency (see **Figure 1**).

In early embryonic development, SALL4 expression in mouse is detected at as early as the two cell stage. At the blastocyst stage, SALL4 expression becomes enriched in the inner cell mass (ICM) and the trophectoderm [17, 20–22]. Reduction of SALL4 in oocytes and ESCs results in early embryo defects, and disruption of both *Sall4* alleles causes embryonic lethality during peri-implantation [23–25]. SALL4 is also expressed in extraembryonic endoderm (XEN) cells, where it participates in cell fate decision by simultaneously activating pluripotency-maintaining factors and silencing endoderm lineage-associated factors such as GATA6, GATA4, and SOX17 [26, 27]. During subsequent stages, heterozygous disruption of *Sall4* allele leads to multi-organ malformations including limb and heart defects, which model human disease [25]. It has been reported that TBX5, a gene encoding a T-box transcription factor, regulates SALL4 expression in the developing forelimb and heart, and interacts with SALL4 to synergistically regulate downstream gene expression [24, 25, 28].

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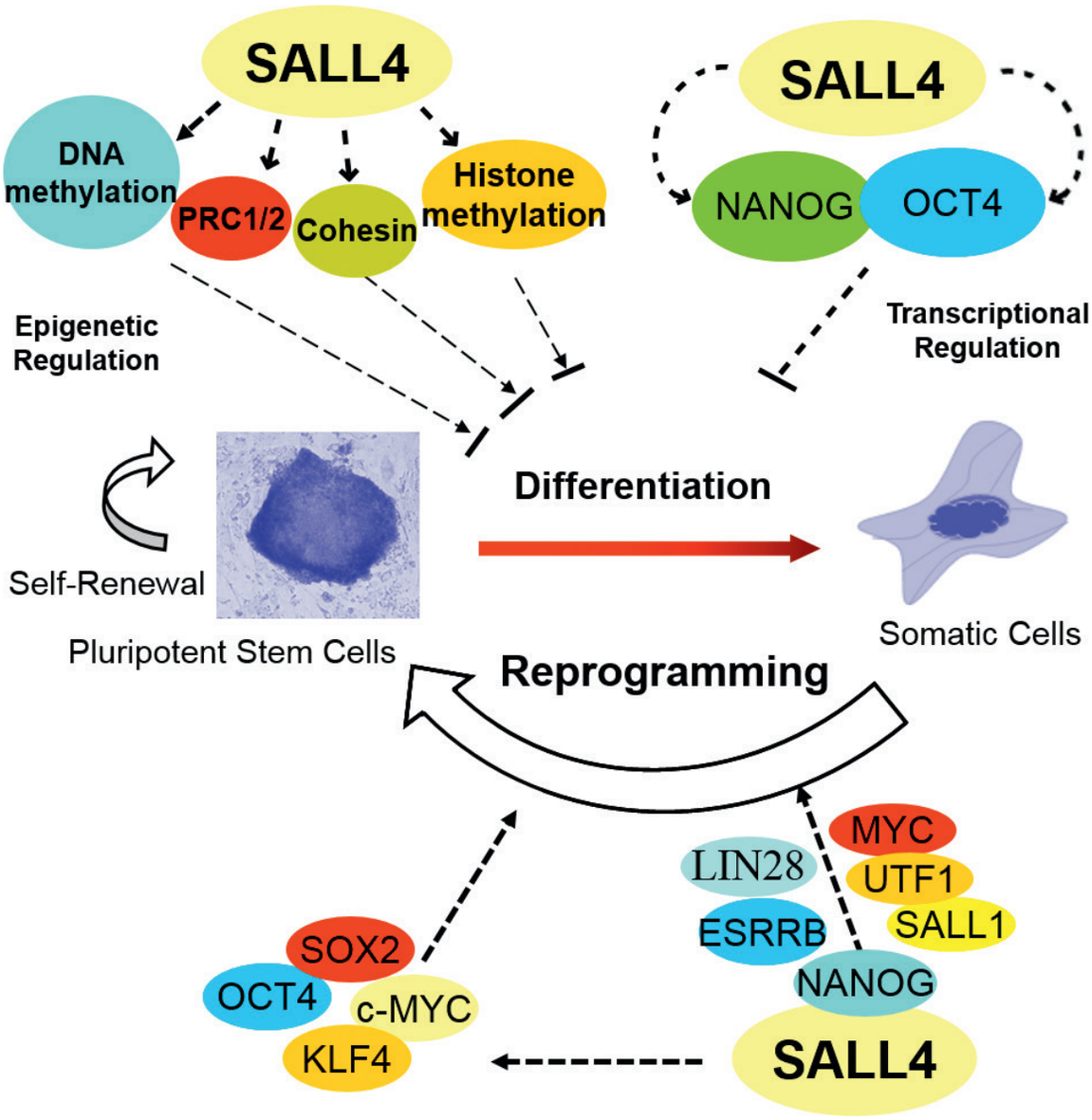

Figure 1. SALL4 plays a variety of regulatory functions in maintaining and/or reprogramming cells to pluripotency. PRC: polycomb-repressive complexes.

2.2. SALL4 is a potent regulator in reprogramming somatic cells to pluripotency

Decreased SALL4 expression in ESCs has been shown to downregulate the expression levels of Oct4, Sox2, Klf4, and c-Myc (OSKM), the four proteins capable of reprogramming murine somatic cells to an induced pluripotent state [18, 29]. Consistently, knockdown of SALL4 in fibroblasts decreased the efficiency of induced pluripotent stem cell (iPSC) generation, while overexpression of SALL4 significantly increased iPSC generation [30, 31]. In a recent study by Shu et al., the GATA family members GATA4 and GATA6 have been found to substitute for OCT4 in mouse somatic reprogramming, and SALL4 is identified as a major target gene of the GATA members [32]. In another study by Buganim et al., ectopic expression of SALL4, NANOG, ESRRB, and LIN28 in mouse fibroblasts generated high-quality iPSCs more

efficiently than the combination of OSKM [33]. Similarly, Mansour et al., showed that the combined overexpression of SALL4 with stem cell factors SALL1, UTF1, NANOG and MYC also replaced exogenous OSK expression and generated chimaera formation- competent iPSC clones [34]. Together, these studies suggest that SALL4 not only plays a role in ESC property maintenance, but its overexpression also drives reprogramming of somatic cells toward a stem cell-like fate (see **Figure 1**).

2.3. SALL4 regulates distinct transcriptional networks in ESCs and XEN cells

SALL4 appears to be unique among the core ESC pluripotency regulators because it is also expressed in non-ESC stem cell fractions where Oct4 and/or Nanog are silenced. These include XEN cells, mesodermal progenitor cells [35], embryonic cardiac progenitor cells [36], fetal liver stem/progenitor cells [27], and adult stem cells such as bone marrow HSCs/HPCs [37]. In these cells, SALL4 regulates downstream networks in a cell type-specific manner. Genome-wide promoter binding assays in murine ESCs and XEN cells revealed that SALL4 regulates disparate gene sets in these cells, and down-regulation of SALL4 targets in the respective cell types induced differentiation [26]. Also consistent with the previous report [18], Sall4, Oct4, Sox2, and Nanog in murine ESCs formed a crucial interconnected autoregulatory network. In XEN cells however, SALL4 regulates the key XEN lineage-associated genes Gata4, Gata6, Sox7, and Sox17 (see **Figure 2**). Moreover, transcription assays revealed that SALL4 regulates

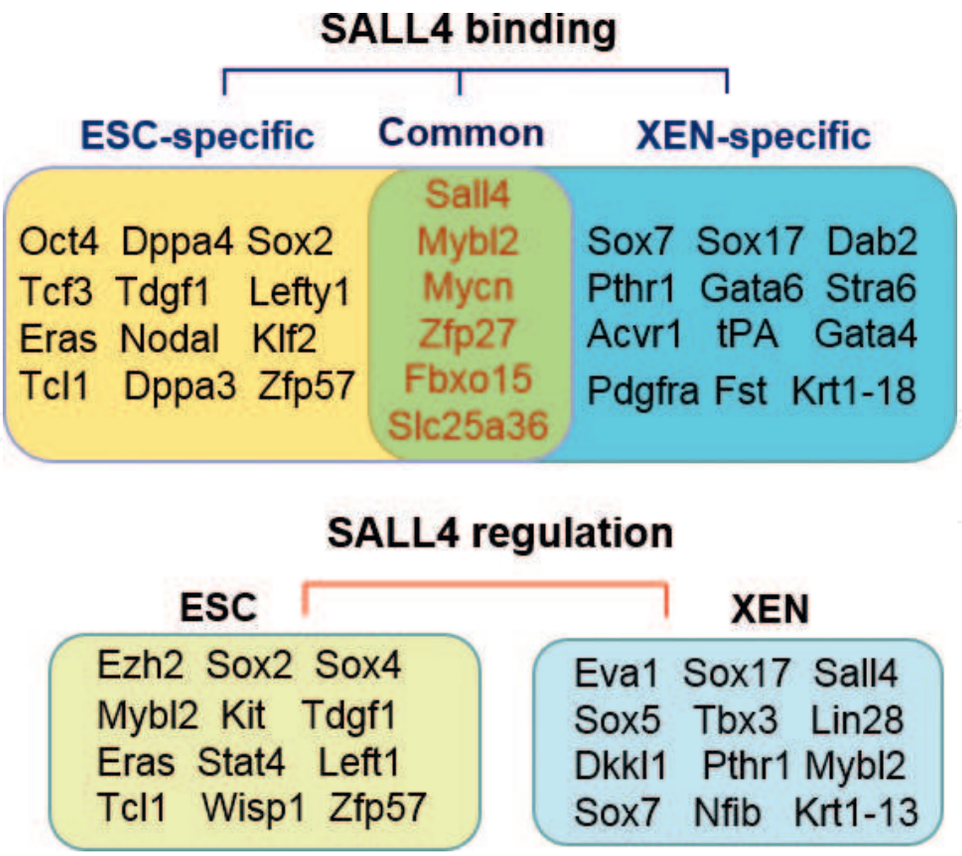

Figure 2. SALL4 binds and regulates distinct target genes in ESCs and XEN cells. Shown are examples of such genes in each cell types. Figure modified from Ref. [26].

the expression of more than half of its binding genes in ESCs, but downregulation of SALL4 did not result in similar expression changes in the majority of these genes in XEN cells [26].

3. Functions of SALL4 and its regulated networks in normal hematopoiesis

3.1. The SALL4 isoforms are robust simulators for HSC/HPC *ex vivo* expansion

In humans and mice, the SALL4 proteins exist in at least three isoforms termed A, B and C, with SALL4A (full length) and SALL4B (lacks a portion of exon2 sequence) being the most studied [38–40]. To date, the function of SALL4C isoform (exon2 sequence spliced out) has not been well characterized. In the human blood system, the cellular expressions of SALL4 isoforms have been originally investigated by immunofluorescence staining and qRT-PCR assays, which revealed that both A and B isoforms are highly expressed in bone marrow CD34+CD38− HSCs, downregulated in CD34+CD38+ HPCs, and absent in CD34− differentiated lineage cells. Similarly, the SALL4 -A and -B isoforms in mouse bone marrows were found selectively expressed in the nuclei of Lin-Sca1+cKit+ (LSK) HSCs. The functions of SALL4 in the self-renewal of HSCs/HPCs have been explored. We and others reported that the SALL4 isoforms are robust stimulators for CD34+ (or CD133+) HSCs/HPCs *ex vivo* expansion, and the SALL4-mediated cell expansion was associated with enhanced cell engraftment and long-term repopulation capacity in transplanted mice [40–44]. In mouse model studies, forced overexpression of the SALL4 isoforms in bone marrow LSK cells likewise leads to sustained cell proliferation, as well as enhanced marrow-repopulating potential *in vivo* [39]. By transcripts assays, the increased HSC/HPC growth was found associated with upregulation of important HSC regulatory genes including HOXB4, NOTCH1, BMI1, RUNX1, CMYC, MEIS1 and NF-YA [39]. Further, in a myeloid progenitor cell line (32D cell) study, overexpression of the SALL4 isoforms blocked granulocyte-colony stimulating factor (G-CSF)-induced granulocytic differentiation, and permitted expansion of undifferentiated cells in the presence of defined cytokines [39, 40]. Thus, the SALL4 isoforms stimulate HSC/HPC proliferation by activating important self-renewal regulators and simultaneously inhibiting cellular differentiation. These studies provide a new avenue for investigating mechanisms of SALL4-regulated HSC/HPC self-renewal and potentially achieving clinically significant expansion of transplantable human HSCs.

3.2. ChIP-on-chip and gene expression assays identified important target genes that are regulated by SALL4

In their study of SALL4 regulated networks in normal hematopoiesis, Gao et al. have sorted human bone marrow and cord blood CD34+ cells, and performed ChIP-on-chip together with gene expression assays . This investigation identified that CD34, RUNX1, HOXA9, and PTEN are SALL4-directed target genes in these cells. In particular, HOXA9 was characterized as a major SALL4 target in normal hematopoiesis . In another study, the polycomb complex protein BMI-1 as a critical SALL4 downstream target has been documented [45]. Chromatin

immunoprecipitation coupled with quantitative PCR (ChIP-qPCR) in the 32D myeloid progenitor cells reveals that SALL4 binds to a specific region of *Bmi-1* gene promoter, and heterozygous disruption of *Sall4* allele significantly reduced BMI-1 expression in bone marrow cells. Further, in transgenic mice that constitutively overexpress human SALL4B, there is upregulated expression of BMI-1, whose levels increase in the progression from normal to preleukemic (myelodysplastic syndrome [MDS]) and leukemic (acute myeloid leukemia [AML]) stages [45].

3.3. SALL4 roles in normal HSC/HPC capacity maintenance

In human CD34+ cell studies, a shRNA-mediated SALL4 knockdown resulted in decreased *in vitro* myeloid-colony-forming ability and impaired *in vivo* engraftment. Further, loss of either SALL4 or its downstream target HOXA9 expression in CD34+ cells shared a similar phenotype. These findings indicate that the role of SALL4 and HOXA9 in normal hematopoiesis is to maintain the HSPCs in an undifferentiated stage with self-renewal capacity [37]. Very recently, the roles of SALL4 in normal hematopoiesis have been further explored using conditional gene targeting approaches in mice [46]. Unexpectedly, wild type *Sall4*$^{f/f}$/CreER^{T2} mice treated with tamoxifen or vav-Cre-mediated (hematopoietic-specific) *Sall4*$^{-/-}$ mice were all healthy and displayed no significant hematopoietic defects, which contrasts to previous findings from human CD34+ cell studies. Reasons for this discrepancy have not been fully addressed. However, it has been speculated that SALL4 may have a redundant role during homeostasis, which can be compensated by other *Sall* gene family members, or *pretreatment* of gene knockdown may not truly reflect the actual performance of gene functions *in vitro* or *in vivo*. On the other hand, some genes may exert aberrant functions only when cells encounter transplantation or replicative stress (see review [47]), and some vav/Cre knockout models may demonstrate hematopoietic defects at late stages [48]. Therefore, it might be necessary to perform serial transplantation and/or stress induction (such as 5-fluorouracil injury) assays with SALL4-deficient cells to fully clarify SALL4 effect and mechanisms in normal HSC capacity maintenance.

4. Functions of SALL4 and its regulated networks in leukemia

4.1. SALL4 is aberrantly expressed in human leukemias

SALL4 is absent in most adult tissues and SALL4 expression in bone marrow is restricted to the rare CD34+ HSCs/HPCs. However, aberrant expression of SALL4 has been detected in various human solid tumors as well as different types of leukemias [49–57]. In patients with MDS, a group of preleukemic hematologic disorders, a high level of SALL4 expression is detected and correlated with high-risk patients with poor survival [58, 59]. In AML cases, our group and others have reported that SALL4 mRNA or proteins are aberrantly expressed in various AML subtypes (ranging from M1 to M5, the French-American-British [FAB] classification), and SALL4 expression is involved in chromosomal instability and associated with disease status and drug treatments [59–65]. SALL4 expression is found significantly higher in AML patients with complex karyotype (equal to or more than three aberrant karyotypes) than that in MDS patients with normal karyotype [63]. In chemotherapy cases, it has been reported that SALL4 has the highest expression level in de novo AML patients which then decreases

in partial remission (PR), and then even lower in complete remission (CR) [61, 62]. Further, SALL4 was found to decrease throughout the treatment process in the drug responsive group but increase in drug resistant group [62]. In other leukemia cases, aberrant SALL4 expression has been reported in ALK positive anaplastic large cell lymphoma (ALK^+ ALCL) [66], B cell acute lymphocytic leukemia (B-ALL), most prominently in B-ALL patients with TEL-AML1 translocation, which is the most common genetic abnormality in pediatric B-ALL [67, 68]. SALL4 expression is also detected in precursor B-cell (but not T-cell) lymphoblastic leukemia/lymphomas [61]. In addition, SALL4 expression has been detected in patient samples from blastic stage of chronic myeloid leukemia (CML), as opposed to the chronic phase, and in samples from CML patients who have achieved complete remission or those who have tyrosine kinase inhibitor resistance [61, 69, 70].

4.2. Role of SALL4 in transgenic model and in MLL-rearranged leukemia

Given the detection of aberrant SALL4 expression in leukemia patients, our research group has previously investigated transgenic mice that overexpress either human SALL4A or SALL4B. Interestingly, all the *SALL4B* mice developed MDS-like features at 2 months of age, and nine of them (53%) progressed to AML. In contrast, the SALL4A mice did not exhibit leukemia formation during the test period [59]. These studies suggest that SALL4B, but not SALL4A, has oncogenic activity in inducing leukemogenesis. In mechanism studies, the SALL4 isoforms were found to bind β-catenin protein, and these factors synergistically enhanced the Wnt/β-catenin signaling pathway. As expected, the expression levels of cyclin-D1 and c-Myc, the two known targets of the Wnt/β-catenin pathway, were both increased in the *SALL4B* mice bone marrow cells. Interestingly, in a recent study, transgenic activation of the SALL4 target β-catenin in osteoblasts, the HSC/HPC niche, also induced MDS and AML development. Notably, these β-catenin mutated mice were anemic at as early as 2 weeks and died before 6 weeks of age, indicating a severe driving event in leukemogenesis [71]. Further in-depth studies are therefore needed to elucidate whether SALL4B in transgenic mice potentially induces leukemogenesis via activating β-catenin in the osteoblastic niche.

Recently, our group explored SALL4 functions in leukemia pathogenesis induced by MLL-AF9, one of the most common mixed lineage leukemia (MLL)-rearranged (MLL-r) oncoproteins found in leukemia patients which is associated with very poor prognosis [72–76]. A previous study showed that SALL4 physically interacts with the MLL wild type protein in regulating HOXA9 expression [77]. In this study, our data revealed that loss of SALL4 in MLL-AF9-transformed bone marrow cells largely disrupted their clonogenic ability in methylcellulose-based medium and in liquid culture, induced markable apoptosis and cell cycle arrest at G1. Consistently, conditional disruption of both *Sall4* alleles in transplanted mice completely blocked leukemia initiation and significantly attenuated pre-existing disease progression [46]. Therefore, these studies suggest that SALL4 is an essential transcriptional regulator in MLL-r leukemogenesis.

4.3. SALL4 regulated pathways in leukemia

Our research group has previously conducted ChIP-on-chip assays with a promyelocytic leukemic cell line NB4 [78]. Analysis of the SALL4-bound genes revealed the most prominent pathways involving WNT/β-catenin, apoptosis, NOTCH signaling, the polycomb complex

protein BMI-1, PTEN, and nuclear factor-kB (see **Figure 3**). When the cells were treated with a SALL4-specific shRNA vector, the expression levels of proapoptotic genes TNF, TP53, PTEN, CARD9, CARD11, ATF3, and LTA were upregulated. In contrast, the expression levels of anti-apoptotic genes such as BCL2, BMI-1, DAD1, TEGT, BIRC7, and BIRC4 (XIAP) are downregulated. In line with the expression studies, reduction of SALL4 also diminished tumorigenicity of leukemic cells in immunodeficient mice. Further, the SALL4 knockdown-induced apoptosis was reversed by ectopic expression BMI-1. In a separate study, SALL4 knockdown in combination with a BCL-2 inhibitor also synergistically increased apoptosis in AML cells. Other studies have reported that SALL4 recruits the nucleosome remodeling and histone deacetylation (NuRD/HDAC) repressive complex to the promoter of *PTEN* and decrease its gene expression [79], while conversely, a SALL4-derived peptide blocking this protein-protein interaction resulted in notable leukemic cell death, and this effect was reversed by treatment of a PTEN inhibitor [80]. In AML differentiation studies, SALL4 expression has also been

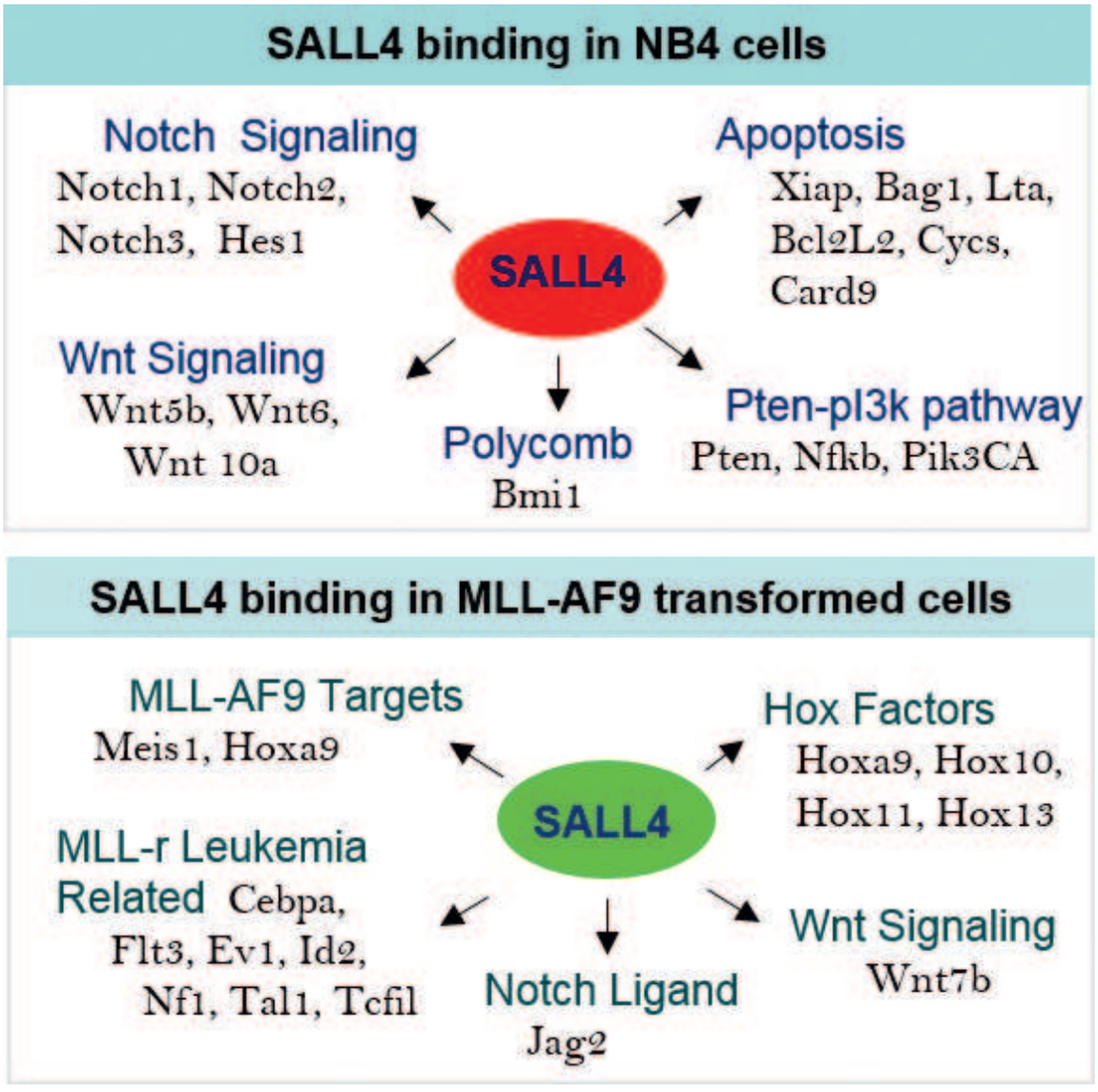

Figure 3. Key signaling pathways bound by SALL4 in NB4 acute promyelocytic and MLL-AF9 transformed leukemic cells.

reported to block all-trans retinoic acid (ATRA)-induced myeloid differentiation in ATRA-sensitive and -resistant AML cells. Further, inhibition of SALL4 and its interacting epigenetic factor LSD1 synergistically promoted ATRA-induced cell differentiation and growth arrest. In mechanistic studies, SALL4 and LSD1 have been found to co-occupy on the ATRA targets *RARβ*, *ID2*, and *CYP26* gene promoters, and cooperatively regulate their expression [81–82].

Recently, our research group also conducted ChIP assays with sequencing (ChIP-Seq) assays with MLL-AF9 transformed murine leukemic cells. This study revealed that SALL4 binds to the key MLL-AF9 target genes *Meis1*, *Hoxa9*; MLL-r leukemia related genes *Cebpα*, *Id2*, *Elf1*, *Evl*, *Flt3*, *Nf1*, *Tal1*, *Tcf7l1*, *Nkx2–3*; the Hox factors *Hoxa-9*, *−10*, *−11*, *−13*; the Notch ligand *Jag2*, and Wnt/β-catenin regulator *Wnt7b* (see **Figure 3** and [46]). mRNA microarrays assays following early *Sall4* deletion identified multiple upregulated genes including cell cycle inhibitors *Cdkn1a (p21)*, *Trp53inp1*; HSC/HPC colony-forming repressor *Slfn2*; and hematopoietic differentiation markers *Col5a1*, *Fyb*, *Irf8* and *Pira6*. In contrast, the TGFβ family genes, *Tgfβ2*, *Tgfβ3*, *Tgfβr3,* and the genes related to chemo-resistance or leukemia aggressiveness, such as *Thbs1*, *Tgm2*, and Ambp were downregulated [46, 83]. In comparison with the mRNA expression data, not many of the ChIP-Seq-identified SALL4 targets were associated with early expression changes. This limited overlap has been considered to be related to the length of time of SALL4 inactivation, the presence of other co-regulators in play, and/or the relatively lower number of genes identified in relevant assays. More detailed studies would help to address these issues.

4.4. SALL4 regulates different downstream networks in normal and leukemic cells

In the SALL4-binding genes identified in NB4 leukemia and those in normal CD34+ cells, less than 20% of the targets were found commonly bound by SALL4. This limited overlap mirrors the findings from ESC and XEN cell promoter binding studies, and further indicates that SALL4 functions in a manner specific to cell type or cell context (see **Figure 4**). Particularly, downregulation of SALL4 expression seems to have an opposite effect on genes involved apoptosis. For example, in leukemic cells, when SALL4 was downregulated along with the apoptotic phenotype, the expression levels of proapoptosis genes TRO

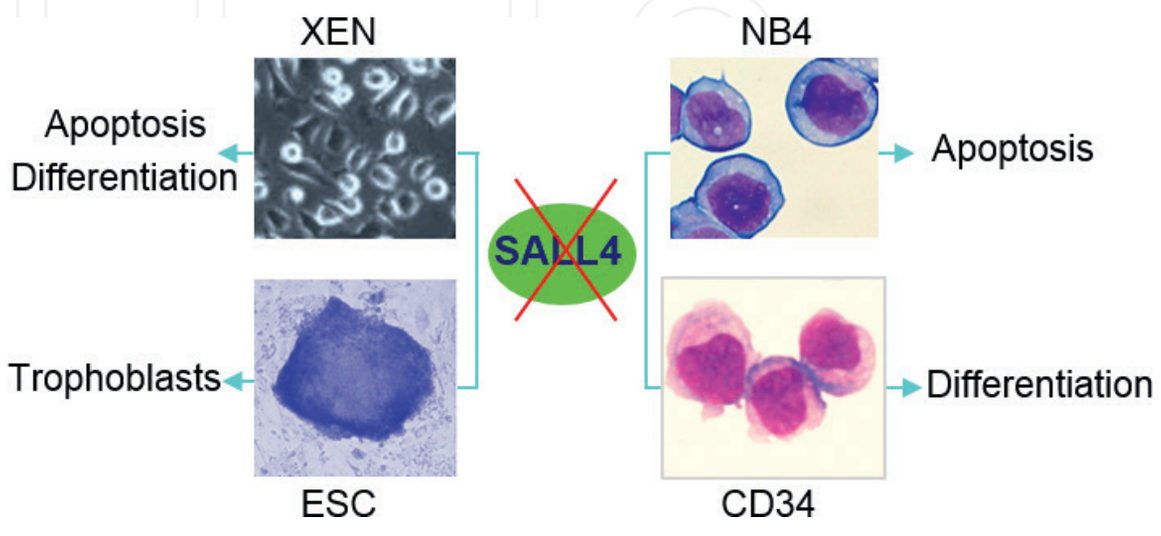

Figure 4. SALL4 functions in a manner specific to cell type or cell context. Shown are main effects following SALL4 knockdown in indicated cell types.

and ABL1 increased, and the expression of anti-apoptosis gene BCL2 decreased. While in CD34+ cells, there was no notable apoptosis with SALL4 knockdown, and the expression of BCL2 increased whereas the expression of TRO and ABL1 decreased. This differential regulatory effect by SALL4 should be helpful in developing SALL4-targeted anti-leukemia strategies to spare normal blood cells.

5. Epigenetic mechanisms involved in SALL4's regulatory functions

5.1. SALL4 interacts with a variety of epigenetic factors to regulate downstream gene expression

So far the reported SALL4-interacting epigenetic factors (see **Figure 5**) include: DNA methyltransferases DNMT-1, -3A, -3B, -3 L, methyl-CpG-binding domain 2 protein (MBD2) [84]; NuRD complex that contains histone deacetylases HDAC1/2 [79]; H3K4 methyltransferase MLL1 [77]; H3K79 methyltransferase DOT1L [46]; H3K36 methyltransferase Wolf-Hirschhorn syndrome candidate 1 (WHSC1) [85, 86]; and lysine-specific histone demethylase LSD1/KDM1A [46, 81, 87]. All of these are critical regulators in normal blood development and are frequent targets for dysregulation in hematological malignancies [88–90], and clinical epigenetic remedies inhibiting such epigenetic factors have been shown effective in treating leukemia [91–93]. In fact, in MLL-AF9-mediated mouse AML studies, genetic disruption of either SALL4, DNMT1, LSD1, or DOT1L likewise blocked leukemia initiation and delayed disease progression *in vivo* [94–96].

By interacting with specific epigenetic factors, SALL4 expression can affect DNA methylation and histone methylation/acetylation status at genes that control hematopoietic differentiation,

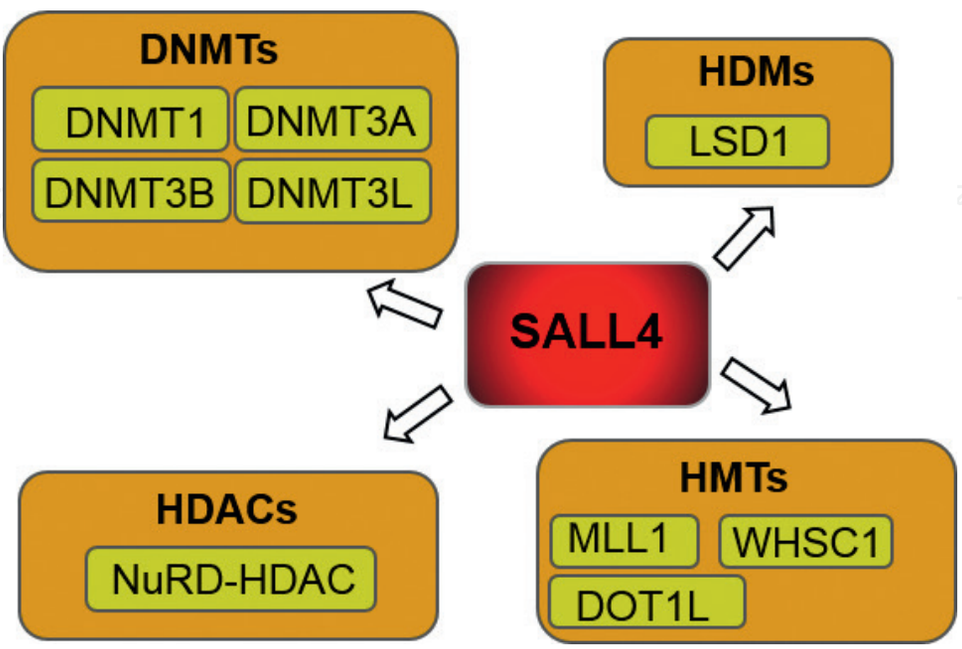

Figure 5. The SALL4-associated epigenetic factors. DNMTs: DNA methyltransferases. HDACs: histone deacetylases. HDMs: histone demethylases. HMTs: histone methyltransferases.

apoptosis, tumor induction or suppression. For example, in NB4 AML cells that were transduced with a lentiviral SALL4 vector, there was an overall increased percentage of DNA methylation at various CpG sites of tumor suppression gene *PTEN*, which co-relates with a downregulated gene transcription [84]. In mouse bone marrow LSK cells, overexpression of SALL4 also induced increased percentage of methylation at the CpG sites of early B-cell factor 1 (*Ebf1*) promoter, as well as the *Sall4* gene promoter itself, which facilitates an undifferentiated cellular status [84]. Similarly, the SALL4 overexpression levels significantly affected LSD1 binding and altered H3K4me2 levels at the promoter regions of tumor necrosis factor (*Tnf*) and differentiation-related genes *EBF1*, *GATA1*, *RARβ*, *ID2*, and *CYP26*, which are associated with relevantly altered gene transcription levels [81, 87]. Also, while SALL4 interacts with the NuRD/HDAC1/2 complex to silence *PTEN* promoter via reduced acetylation of histone H3 at its binding sites, the SALL4-derived peptide blocks this interaction and leads to reactivated PTEN expression. Additionally, in the 32D myeloid progenitor cells following lentiviral SALL4 transduction, the H3K4me3 and H3K79me2/3 levels at *Bmi1* promoter regions were increased [45]. In MLL-AF9 leukemia studies, the expression levels of SALL4 also affected LSD1 and Dot1l binding and relevant H3K4me3 and H3K79me3 amounts at the promoter regions of *Meis1* and multiple HOX family genes in bone marrow cells [46, 77, 79].

5.2. SALL4 regulated epigenetic modification programs are cell type-dependent

Consistent with the findings from SALL4 genome-wide promoter binding and relevant expression assays, SALL4-regulated epigenetic modification programs are also strictly dependent on the cellular context. As reported, SALL4-bound genomic loci in murine ESCs are largely enriched for the activating marker H3K4me3, which indicates an association of SALL4 with non-repressed genes. In XEN cells, however, SALL4-binding loci displayed significantly less H3K4me3 enrichment. Instead, most of these regions are either accompanied with H3K27me3 or lacking both H3K4me3 and H3K27me3, the "epi-markers" frequently associated with gene repression [26]. In our MLL-AF9 leukemia model studies, SALL4 has been shown to recruit DOT1L and LSD1 to *Meis1* and HOX family gene promoters and modulate their H3K79me2/3 and H3K4me3 levels [46]. The previously demonstrated SALL4-MLL interaction may contribute to the observed HEK4me3 changes. However, in some non-MLL-r human AMLs, the DOT1L-regulated H3K79 methylation may not play a role, and it has been reported that administration of DOT1l inhibitors sensitized chemotherapy in MLL-r but not in non-MLL-r AML cells [97]. Further, the DOT1L recruitment to MLL-AF9 has been associated with the level of leukemic transformation [98–100]. Therefore, one may anticipate that SALL4 differentially interacts with individual epigenetic factors to exerting a disease/subtype–dependent regulatory effect. This concept, if proven true, should further facilitate the development of SALL4-based disease subtype–specific anti-leukemia strategies.

6. Conclusions

Abnormal expression of SALL4 has been frequently detected in different types of human leukemias and associated with disease status and drug treatments. On the other hand, proper manipulation of SALL4 expression might be useful in achieving clinically significant

expansion of transplantable human HSCs. Therefore, understanding how SALL4 mechanisms maintain normal HSCs/HPCs vs. leukemic cells will facilitate development of newer, more efficient therapies in clinic.

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Conflict of interest

The authors declare that they have no conflicts of interest with the contents of this article.

Acronyms and abbreviations

ESC	embryonic stem cell
HSPs/HPCs	hematopoietic stem/progenitor cells
C2H2-ZF	Cys2His2 zinc finger
PRC	polycomb-repressive complexes
ChIP-on-chip	chromatin immunoprecipitation followed by microarray hybridization
ICM	inner cell mass
XEN	extraembryonic endoderm
iPSC	induced pluripotent stem cell
LSK	lineage- Sca-1+ c-kit+
G-CSF	granulocyte-colony stimulating factor
MDS	myelodysplastic syndrome
AML	acute myeloid leukemia
FAB	the French-American-British classification
ALK+ ALCL	ALK positive anaplastic large cell lymphoma
B-ALL	B cell acute lymphocytic leukemia
CML	chronic myeloid leukemia
MLL-r	mixed lineage leukemia (MLL)-rearranged

NuRD/HDAC	nucleosome remodeling and histone deacetylation
ATRA	all-trans retinoic acid
ChIP-Seq	ChIP assays with sequencing
MBD2	methyl-CpG-binding domain 2 protein
WHSC1	Wolf-Hirschhorn syndrome candidate 1
Ebf1	early B-cell factor 1

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