ZEB Proteins in Leukemia: Friends, Foes, or Friendly Foes?

Bieke Soen\textsuperscript{1,2}, Niels Vandamme\textsuperscript{1,2}, Geert Berx\textsuperscript{1,2}, Jürg Schwaller\textsuperscript{3}, Pieter Van Vlierbergh\textsuperscript{2,4}, Steven Goossens\textsuperscript{1,2,4}

Correspondence: Steven Goossens (e-mail: Steven.Goossens@ugent.be).

Abstract

ZEB1 and ZEB2 play pivotal roles in solid cancer metastasis by allowing cancer cells to invade and disseminate through the transcriptional regulation of epithelial-to-mesenchymal transition. ZEB expression is also associated with the acquisition of cancer stem cell properties and therapy resistance. Consequently, expression levels of ZEB1/2 and of their direct target genes are widely seen as reliable prognostic markers for solid tumor aggressiveness and cancer patient outcome.

Recent loss-of-function mouse models demonstrated that both ZEBs are also essential hematopoietic transcription factors governing blood lineage commitment and differentiation. Interestingly, both gain- and loss-of-function mutations have been reported in multiple hematological malignancies. Combined with emerging functional studies, these data suggest that ZEB1 and ZEB2 can act as tumor suppressors and/or oncogenes in blood borne malignancies, depending on the cellular context. Here, we review these novel insights and discuss how balanced expression of ZEB proteins may be essential to safeguard the functionality of the immune system and prevent leukemia.

ZEB1 and ZEB2: 2 structurally related E-box-binding transcription factors

The ZEB (Zinc finger E-box-binding homeobox) protein family of transcription factors was discovered in Drosophila melanogaster and consists of 2 structurally conserved multidomain proteins, ZEB1 (originally called ZFHX1A, TCF8, or 8EF1) and ZEB2 (originally called ZFHXB1 or SIP1).\textsuperscript{1,2} Both ZEBs contain an amino-terminal (NZF) and carboxy-terminal zinc finger cluster (CZF), which enables them to bind regulatory DNA sequences in their target promoters. Each zinc finger cluster binds an independent 5'-CANNTG-3' motif, which overlaps with the core of an E2-box sequence,\textsuperscript{3-5} a binding site for transcription factors of the basic helix-loop-helix (bHLH) family. ZEBs can repress target gene transcription through the recruitment of the CtBP corepressor complex via their CtBP interaction domain (CID).\textsuperscript{6} Additional conserved domains were documented by which ZEBs can recruit other transcriptional complexes to their target promoters. For instance, the amino-terminus of both ZEBs can bind the Nucleosome Remodeling and Deacetylase complex (NuRD, also known as the Mi-2 complex).\textsuperscript{7-9} Also, a SMAD Binding Domain (SBD) is located between the NZF and the central homeobox domain.\textsuperscript{8} Although this SBD is highly conserved and both ZEBs can directly interact with SMADs, they may have an opposite downstream effect\textsuperscript{9}. ZEB1 synergizes with SMAD proteins to activate SMAD-mediated transcription, while ZEB2 seems to inhibit SMAD-mediated transcription. It is believed that this opposite effect is regulated by the differential recruitment of ZEB1/2-specific coactivators/repressors, which may be tissue-specifically expressed. Initially, the N-terminal region of ZEB1, but not ZEB2, was documented to interact with p300 and P/C/AF. This differential recruitment of P/C/AF may switch ZEB1 from a repressor to an activator through the displacement of CtBP1 from the CID.\textsuperscript{9,10} Later, others demonstrated that, depending on the experimental context, ZEB2 and ZEB1 are equally potent to bind p300 and P/C/AF.\textsuperscript{11} This suggests that other mechanisms/cofactors are contributing to the differential ZEB1/2-specific effects on SMAD-mediated transcription.

Based on the extensive and continuously growing list of interaction partners and putative downstream targets, one can expect that ZEBs have very pleiotropic functions, which largely depend on the cellular context. Indeed, the chromatin status and the presence/absence of tissue-specific interaction partners strongly influence their role as a transcriptional repressor/
ZEBs are master regulators of (cancer) cell plasticity

ZEBs during EMT

ZEB proteins are primarily known as inducers of epithelial-mesenchymal transition (EMT), a reversible multistep process during which polarized epithelial cells undergo a morphological switch to become motile mesenchymal cells that have lost their polarity.2,2 ZEBs orchestrate EMT by the direct repression of epithelial genes involved in cellular adhesion and cytoskeleton (re)organization,11,14 and subsequent direct or indirect upregulation of mesenchymal genes.3,16 This epithelial-to-mesenchymal cell plasticity is essential at various stages of embryonic development, but also often seen aberrantly activated in cancer cells.11 Induced expression of ZEB1 or ZEB2 in xenotransplanted epithelial cancer cells endows them to invade and disseminate from the primary tumor site. These circulating tumor cells will then need to undergo a reversed process called mesenchymal-epithelial transition or MET to colonize a distant tissue and grow out as a metastasis.

In addition, EMT-inducing transcription factors (EMT-TF), like ZEB1 and ZEB2, are more than "only" regulators of cancer cell invasion and they dictate multiple other steps during cancer initiation and progression.3,2,11 Increased ZEB expression has been correlated with the acquisition and/or function of cancer stem cell (CSC) properties.11-20 CSCs or tumor-initiating cells have the potential to self-renew and form secondary tumors when transplanted into immune deficient or syngeneic mice. The molecular mechanisms controlled by EMT-TF that induce these CSC properties remain elusive until today.3,11,21 In addition, a large body of literature suggests that CSC subpopulations are responsible for emerging residual subpopulations following radiotherapy, chemotherapy, or other targeted therapies. Also EMT-TF-driven cancer cell plasticity facilitates drug adaptation and protects the cancer cells against genotoxic insults and therapy-induced stress via enhanced cell survival, induced DNA damage repair,21,22 antiproliferative mechanisms,23 and increased expression of genes encoding drug-metabolizing enzymes and drug-transporters.24 As such, ZEBs can act as important regulators of therapy resistance.

Next to ZEB proteins, also SNAI and TWIST transcription factors have been demonstrated to catalyze similar EMT phenotypes in the context of solid tumors.12,17 Consistent results in cancer cell lines suggest cooperativity between the different EMT inducers with complex regulatory feedback mechanisms involving Notch signaling and the miR-200 family.3,2

To conclude, the pivotal roles of the EMT modulators in solid tumor progression have been extensively documented. It is therefore no surprise that ZEB expression levels and the expression of their targets are used in the clinic as reliable prognostic markers for solid tumor aggressiveness and poor patient outcome.

Emerging roles of ZEB proteins in hematopoiesis

Gain- and loss-of-function mouse models revealed that ZEB proteins also play pivotal roles in cellular plasticity of other cell lineages, including the hematopoietic lineage.

Next to the full Zeb1 knockout, which is perinatal lethal due to skeletal defects,20,21 other Zeb1 mutant mouse models expressing truncated C-terminal ZEB1 deletions lacking the CZF domain have been generated.20-23 The first one, Zeb1ACfl mice have a profound thymic atrophy with spontaneous development of CD4+ T-cell lymphoma at older age.29 Their hypoplastic thymus show an increased proportion of CD4/CD8 double negative cells and a drastic reduction in double positive cells, suggestive of an early partial block in T-cell development.27 The few Zeb1ACfl thymocytes that can circumvent this early block and differentiate into mature T-cells are skewed toward the CD4+ T-cell lineage. In line with this, previous work has shown that ZEB1 can directly regulate CD4 expression by competing with transcriptional activators E12 and HEB for the binding to an E-box-containing enhancer of the CD4 promoter.28 The Zeb1cellophane mice, the second Zeb1 mutant model with a C-terminal truncation, are also characterized by hypoplastic thymus that display an early T-cell differentiation block. Detailed analysis of other lineages suggested B-cell and NK-cell maturation defects in the spleen of these Zeb1 mutant mice.28 Until now, it is unclear whether these defects are cell autonomous or due to aberrant paracrine signaling. Similarly, it remains to be established whether these truncated ZEB1 versions are true loss-of-function mutations and/or can act in a dominant negative manner. A recently generated conditional Zeb1fl/fl knockout mouse line31 will allow further investigation of the role of Zeb1 in specific hematopoietic lineages.

The role of Zeb2 in hematopoiesis has been studied with conditional loss- and gain-of-function mouse models.32,33 Inactivation of Zeb2 in the hematopoietic lineage resulted in vast multilineage differentiation defects, associated with an accumulation of stem and progenitor cells and a significant drop in fully matured functional blood cells.34,35 Differentiation defects appeared at early and later stages of hematopoiesis affecting most lineages, except the granulocytes. These studies were extended by using more lineage-restricted Cre-lines allowing to demonstrate essential roles for ZEB2 for NK-cell maturation,36 terminal differentiation of CD8+ cytotoxic T-cells,37,38 and dendritic cell (DC) development.39 In addition, strong evidence suggests that ZEB2 is also important for Langerhans cell40 and mast cell maturation/activation,41 although the latter is purely based on in vitro work and the relevance of these observations will have to be confirmed in vivo. Altogether, these studies suggest that ZEB2 is a transcription factor that controls lineage commitment and fidelity at various stages of hematopoiesis. In addition, ZEB2 is crucial for proper immune functioning and loss of Zeb2 can lead to defective response to pathogens.

Based on the observed complementary expression patterns of Zeb1 and Zeb2 mRNAs during embryonic development and aggravated phenotypes in compound Zeb1/Zeb2 loss-of-function mice, it was suggested that ZEB transcription factors may have partly overlapping, compensatory functions.42 Remarkably, we observed that Zeb1 and Zeb2 mRNA expression is also mostly mutually exclusive during hematopoietic differentiation, except in the hematopoietic stem and multipotent progenitor cell (HSPC) compartment where Zeb1 and Zeb2 are co-expressed at moderate levels (Fig. 1). Once lineage commitment is initiated, counter-oscillating levels of both mRNAs are observed with fast Zeb1/2 switching at distinct cell fate decision points. In addition, most of the hematopoietic differentiation defects seen in the Zeb1/2 loss- or gain-of-function mouse models occur exactly at these decision points.
checkpoints (Fig. 2, examples are shown for the T-cell lineage [upper panel] and dendritic cell lineage [lower panel]). Based on these observations, one could hypothesize that oscillations of ZEB levels may control hematopoietic differentiation. At this point, the molecular mechanisms that control such a Zeb mRNA switching remain largely unexplored. However, negative feedback mechanisms via the miRNA-200 family might partially explain these Zeb1/2 oscillations. Indeed, miRNA-200 family members are able to inhibit expression of ZEB1/2 at the post-transcriptional level by binding to highly conserved target sites in their 3’-untranslated region. In addition, ZEBs are also able to transcriptionally repress the miRNA-200 family, suggesting a negative feedback loop that can fuel this ZEB1/2 switching. Interestingly, also in non-hematopoietic cell lineages, including the melanocytes, a similar oscillation between Zeb1/2 mRNAs has been described. Of note, this concept has also been suggested for other protein families, including the GATA2/GATA1 switch as an important driver of molecular development. However, this ZEB switching hypothesis does not exclude that ZEB1 and ZEB2 could also have family member-specific functions, which may be dependent on the cellular context.

**Figure 1.** Zeb1 and Zeb2 are mostly mutually exclusive expressed during hematopoietic differentiation. The figure is based on expression data available via http://servers.binf.ku.dk/bloodspot/. CLP = common lymphoid progenitor, GMP = granulocyte monocyte progenitor, HSC = hematopoietic stem cell, IgM+ SP = IgM+ positive side population, MEP = megakaryocyte erythroid progenitor, MPP = multipotent progenitor, PreB = Pre-B cell, ProB = Pro-B cell, pro-NK = natural killer cell progenitor, T-CD4 = T-cell CD4+ single positive, T-CD8 = T-cell CD8+ single positive, T-DN = T-cell CD4+ CD8- double negatives, T-DP = T-cell CD4+CD8+ double positives.

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**Source:** BloodSpot.eu (DOI: 10.1093/nar/gkv1101)
Genetic alterations of ZEBs in leukemia and lymphoma

Based on the importance of ZEB expression during normal hematopoiesis, and their pleiotropic roles in progression of solid cancers, it is expected that ZEB1 and ZEB2 could also play pivotal roles in hematologic malignancies. Indeed, both genetic and in vivo functional studies indicate that ZEBs can act, depending on the lineage, as oncogenic drivers and/or tumor suppressors (Fig. 3).

ZEBs in T-cell malignancies

T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive hematological cancer of thymic T-cell progenitors that gradually accumulate epigenetic and genetic changes, leading to a block in differentiation, increased survival and proliferative expansion of a malignant clone. Over the last decade, the prognosis of T-ALL has gradually improved with the introduction of intensified chemotherapy. However, the outcome of T-ALL patients with primary resistant or relapsed disease remains poor.

A rare (0.2%; 2/1084 patients), but recurrent, t(2;14)(q22;q32) translocation involving the ZEB2 and BCL11B loci has been identified in early T-cell progenitor ALL (ETP-ALL), a heterogeneous subgroup of T-ALL with a unique gene expression profile similar to that of the most immature ETPs. It was hypothesized that this translocation retains ZEB2 expression during T-cell commitment leading to a differentiation block and leukemic transformation. Hematopoietic-specific Zeb2
overexpression in the mouse was sufficient to spontaneously develop T-ALL with an immature Ly11+ expression profile, with profound similarities to the human disease. Overexpression of Zeb2 resulted in increased expression of the interleukin-7 receptor (IL7R) and aberrant activation of the IL7R-JAK/STAT signaling pathway. In addition, ZEB2 overexpression was associated with the acquisition of enhanced leukemia stem cell properties. Activating IL7R mutations are recurrently found in ETP-ALL patients. In addition, a similar immature ETP-ALL leukemia initiation with increased self-renewal was observed after overexpression of a gain-of-function mutant variant of IL7R in p19Arf−/− mouse hematopoietic progenitors.

In contrast to ZEB2, ZEB1 seems to act as a tumor suppressor in T-cell derived hematological neoplasms. ZEB1 has been identified as an essential downstream mediator of the LMO2 oncogene in T-ALL. In line with this, Zeb1 mutant loss-of-function mice showed drastic defects in early T-cell development and spontaneously develop T-cell lymphoma/leukemia with a median onset of 6 months, similar to what has been observed in LMO2 and ZEB2 overexpressing mice. Combined, these data suggest opposing roles for ZEB1 and ZEB2 in T-ALL initiation/progression.

Furthermore, expression analysis of genes mapped within a common (34.6% of patients) breakpoint cluster in the 10p11.2 region of adult T-cell leukemia/lymphoma patients (ATLL), suggested that ZEB1 may act also as a tumor suppressor in these patients. ATLL is a peripheral T-cell malignancy with a mature CD4+ immunophenotype. Most ATLL cell lines and primary cells display low mRNA expression levels of ZEB1, as a consequence of either chromosomal translocations with heterozygous deletion, intragenic mutations, or epigenetic dysregulation. Mechanistically, low ZEB1 levels may result in resistance to TGFβ1-mediated growth arrest. Binding of the TGFβ1 ligand to its receptor activates its kinase activity, leading to phosphorylation of receptor-associated SMAD proteins. These phospho-SMADs accumulate in the nucleus as dimers, and in conjunction with other transcription factors like ZEB1 and ZEB2, they bind regulatory elements within their target gene promoters. As an example, TGFβ1 stimulation induces cell cycle arrest in various tumor cell types via the direct upregulation of the cyclin-dependent kinase inhibitor p21. ATLL cells with low ZEB1 levels appear to be resistant to these antiproliferative effects of TGFβ1. Overexpression of ZEB1 restored the TGFβ1-mediated growth suppression in these cells, associated with increased p21 expression. Mechanistically, ZEB1 expression, and its direct binding to the phospho-SMAD3 complex, was demonstrated to be essential for the recruitment of this complex to the SMAD-Response Element within the p21 promoter.

Aberrant ZEB1 expression has also been linked to 2 other lymphoproliferative T-cell disorders: mycosis fungoides and Sézary syndrome. Mycosis fungoides is a cutaneous T-cell lymphoma (CTCL) developing from clonally expanded skin-homing CD4+ T-cells. Sézary syndrome can arise de novo, but mostly occurs in patients with mycosis fungoides and can be considered as the leukemic variant of this disease. Several genetic alterations targeting ZEB1 have been reported in both forms of CTCL (up to 65%), including translocations, mutations, and both heterozygous and homozygous deletions. Loss-of-ZEB1 expression or function has been associated with the pathogenic role of IL15 signaling in CTCL. Either loss-of-ZEB1 expression, mutation or hypermethylation of the ZEB1 binding sites in the IL15 promoter resulted in a vast upregulation and activation of oncogenic signals. Altogether, these loss-of-function alterations of ZEB1 suggest that the transcription factor acts as a key tumor suppressor in peripheral T-cell lymphoma.
ZEBs in B-cell malignancies

Altered ZEB1 expression and mutations have been associated with 2 types of B-cell malignancies, namely mantle cell lymphoma (MCL) and diffuse large B-cell lymphoma (DLBCL). MCL is an infrequent subtype of non-Hodgkin B-cell lymphoma (B-cell NHL) with a poor response to chemotherapy. 68,69 The molecular hallmark of this disease is overexpression of cyclin D1 due to the chromosomal t(11;14)(q13;q32) translocation. Half of MCLs display constitutive active Wnt signaling with nuclear localization of beta-catenin and concomitant high expression of ZEB1. Downregulation of ZEB1 expression in MCL cell lines reduced their tumor growth capacity in mouse xenograft models. In addition, the cell lines with reduced ZEB1 expression were more sensitive to chemotherapeutics, associated with a differential expression of drug influx/exflux transporters, and genes involved in cell survival/apoptosis. 70 Therefore, this study suggested that ZEB1 could serve as a potential predictive biomarker and putative therapeutic target in MCL.

DLBCL is the most frequent form of adult NHL. 65 ZEB1 can be considered as an oncogene in DLBCL for several reasons. First, strong nuclear immunohistochemical staining for ZEB1 was associated with an adverse 3-year overall survival of DLBCL patients compared to those with no or weak nuclear ZEB1 staining. 71 Next to this, higher levels of the miR-200 family, a known negative regulator of ZEB1 mRNA expression levels, results in a less aggressive behavior of this disease. Helicobacter pylori positive gastric DLBCLs, which typically show lower ZEB1 expression, have less lymph node metastasis, better response to chemotherapy, and are less aggressive. This last subgroup is also characterized by higher expression levels of BCL6, a known predictor of better prognosis in DLBCL and a direct target of ZEB1. 72 Combined, these expression data suggest that ZEB1 may act as an oncogene in these 2 B-cell NHL forms.

Interestingly, loss and not gain of 2q22.3, the genomic region spanning the ZEB2 locus, has been recurrently (18.75%) observed in B-cell lymphoma patients. 75 In addition, rare but recurrent (7.2%) somatic ZEB2 point mutations were found in B-cell precursor acute lymphoblastic leukemia (B-ALL). B-ALL is the most common childhood malignancy that initiates in the bone marrow (BM) with oncogenic transformation of B-cell progenitor cells. In all 5 reported B-ALL patients with mutant ZEB2 locus, a single AA mutation specifically affected the carboxy-terminal Cys2His2 Zn fingers, which have proven to be essential for the DNA binding and E-box recognition capacity of ZEB2. 6 Notably, all of these patients had a very similar expression profile, associated with deregulated expression of ERG6,77 (https://pecan.stjude.org/proteinpaint/ZEB2). The fact that these mutations are uncommon in other B-ALL subtypes, suggests that loss of the ZEB2 DNA binding capacity may be involved in the progression of this disease, which comprises up to 7% of all B-ALLs. The same laboratory also reported a ZEB2-PDGFRB translocation in a B-ALL patient. In this translocation event, the carboxy-terminal zinc finger domain of ZEB2 is missing and in frame fused to the catalytic domain of the Platelet-derived Growth Factor Receptor-B 77,78 (https://pecan.stjude.org/proteinpaint/ZEB2). However, no additional data are available whether this chimeric protein is expressed, functional and whether it contributed to the disease progression. The notion that ZEB2, or a mutant version, may play an important role in the initiation and/or progression of B-ALL is further supported by the observed high occurrence of viral insertions at the Zeb2 locus in 2 independent retroviral mutagenesis screens using mouse models that are predisposed to develop spontaneously B-ALL, the CALM-AF10 transgenic and heterozygote Pax5-/-/ mice. However, no information is available whether these viral integrations result in loss- or gain-of-Zeb2 functions.

Combining the mutation data of the human patients and the mutagenesis screenings in the B-ALL mouse models, we hypothesize that ZEB2 acts as a tumor suppressor in B-cell malignancies, in contrast to ZEB1, which seems to act as an oncogene. More research will be necessary with conditional Zeb1/2 gain/loss-of-function mouse models using a B-cell restricted Cre line to further test this hypothesis.

ZEBs in myeloid malignancies

Acute myeloid leukemia (AML) is a clinically and genetically heterogeneous malignancy, characterized by uncontrolled accumulation of immature myeloid cells mostly in the blood and the BM of the patient. This accumulation of blast cells in the BM interferes with normal hematopoiesis and leads to a general deficiency of hematopoietic cells. AML is understood as the product of a rather limited number of genetic alterations including balanced chromosomal translocations. The MLL1 histone 3 lysine 4 (H3K4) methyltransferase (aka KMT2A, ALL1, TRX) has been found involved in over 100 leukemia-associated rearrangements of which the MLL-AF4, MLL-ENL, and MLL-AF9 are among the most prevalent. 31 Several MLL fusions have been shown to be potent oncogenes in vitro and in vivo in murine as well as in human cells. 82,83 Recently, a conditional mouse model was used to address the role of the cellular origin of MLL-AF9* AML in the biology and clinical outcome of the disease. Activation of MLL-AF9 in long-term hematopoietic stem cells (LT-HSC) induces a particularly invasive and chemoresistant disease. 84 Strikingly, about 10% to 20% of human AMLs express a very similar gene signatures like LT-HSC-derived MLL-AF9 AMLs in mice, which is characterized by expression of high Zeb1 mRNA levels. Chromatin immunoprecipitation (ChIP) experiments suggested that Zeb1 is a direct target of the MLL-AF9 fusion. Interestingly, knockdown of Zeb1 expression compromised the invasive behavior of MLL-AF9 AML cells in vitro and in vivo. Notably, LT-HSC-derived MLL-AF9 AML cells were also characterized by increased expression of many genes regulating migration and invasion, and thus showing at least at the transcriptional level some similarities to that of solid cancer cells undergoing EMT.

In addition to ZEB1, increasing evidence also suggests an important role for ZEB2 in AML development. An AML patient was found with tumor cells carrying a similar t(2;14)(q22;q32) translocation, as earlier identified in immature T-ALL, involving the ZEB2 and BCL11B loci. 85 In the resulting ZEB2-BCL11B fusion transcript, the first 19AA of BCL11B are replaced by the first 24AA of ZEB2. As all functional domains of BCL11B are retained in this fusion product and the inverse BCL11B-ZEB2 transcript could not be detected, the authors concluded that the principal result of this translocation is aberrant expression of BCL11B controlled by the ZEB2 promoter/enhancer. Interestingly, other genetic events driving BCL11B overexpression have been reported in AML, further reinforcing the putative oncogenic role of BCL11B in the myeloid lineage. 56,87 These observations are in sharp contrast with the proposed role of BCL11B in T-ALL as a tumor suppressor. These data therefore suggest that a similar genetic aberration, t(2;14)(q22;q32), can drive leukemic transformation both in the myeloid as well as the lymphoid lineage. In the case of AML, BCL11B overexpression most probably drives...
malignant transformation whereas retained ZEB2 expression during T-cell commitment is most probably the oncogenic driver in the case of immature T-ALL.18 Although the authors mainly focused on the role of BCL11B, it should be noted that ZEB2 mRNA levels in this t(2;14)(q22;q23) positive AML patient were normal, and most likely compensated by the unaffected allele via downregulation of the miR-200 family.88 More recent experiments convincingly showed that ZEB2 expression is essential for maintenance of leukemic growth of AML.88,89

Using an in vitro genome-wide shRNA screening method, followed by an in vivo secondary screen using a murine AML model driven by retroviral expression of an MLL-AF9 fusion, the authors identified ZEB2 as an essential gene for AML progression. Further molecular analysis demonstrated that ZEB2 represses transcription of genes important in myeloid differentiation. Consequently, ZEB2 depletion in AML cells will force differentiation of the leukemic cells. Interestingly, the notion that AML cells depend on sustained ZEB2 expression was recently confirmed by a large-scale deep RNAi screen that unraveled cancer dependencies in an extensive series of human tumor cell lines, including AML36 (Fig. 4).

In a recent study, recurrent focal deletions of ZEB2 were found in pediatric AML patients (6.6%; 13/197 patients).91 Unfortunately, no expression data were presented in this study to conclude whether these single-allele mutations indeed resulted in loss-of-ZEB2 expression and/or functionality.

Similarly, whole genome sequencing identified ZEB2 putative loss-of-function mutations in 16% of blastic plasmacytoid dendritic cell neoplasm (BPDCN) patients.92 BPDCN is a very rare and aggressive myeloid neoplasm originating from precursors of a specialized subset of DCs93–95 with no defined standard of care.

ZEBs in pathogen-induced hematologic malignancies

ZEB2 expression has been shown to be essential for differentiation, maturation, and/or function of CD8+ cytotoxic T cells (CTLs) and NK cells, 2 types of immune cells involved in antiviral host defense.36,38 Interestingly, recurrent deletions of the ZEB2 locus are significantly enriched in the hepatitis C virus (HCV)-related NHL patients.75,96 HCV is a known risk factor to develop B-cell lymphomas. The most convincing evidence is the observation of B-lymphoma regression after HCV eradication by antiviral therapy.

This was further supported by the fact that viral integrations at the Zeb2 locus are sufficient to induce leukemia in mice haplosufficient for Pax5.79,80 This Pax5−/− mouse model spontaneously develops B-ALL only in a conventional animal house and not under specific pathogen-free conditions suggesting that exposure to infectious agents can act as a trigger for the development of B-ALL.97 The expression of ZEB2 may be essential for the functionality of the innate and adaptive immune system to efficiently eradicate these pathogens, but also infected (and partially) transformed cells from the body, before they can develop into an overt lymphoma/leukemia. We recently demonstrated that ZEB2 also plays a pivotal role in the immunosurveillance and clearance of melanoma cells after transplantation in syngeneic mice,36 suggesting it could serve as a more common mechanism also outside of hematologic malignancies.

Also, altered expression of ZEB1 has been seen in a few pathogen-induced leukemia/lymphoma subtypes, like H pylori positive gastric DLBCLs71 and ATLL, which is associated with HTLV-1 infection.29 Infections typically occur around birth and HTLV-1 carriers have a cumulative risk of 2.5% to develop ATLL with an average latency of 55 years, suggesting the need for extra tumorigenic events. However, no studies have been reported yet that specifically investigated the role of ZEB1 in the defense system of the body against infectious agents.

It has been estimated that over 90% of the world’s population is carrying the Epstein-Barr herpes virus (EBV) usually acquired by an infection, often asymptomatic, during childhood. EBV has been linked to many types of malignancies including several epithelial cancers and some B-cell malignancies, such as Burkitt and Hodgkin lymphoma. EBV causes either a latent infection.
which is maintained stably in the host or a lytic phase with active production of viral particles and killing of the host cell. This latent-lytic switch can be initiated by expression of the viral immediate-early BZLF1 gene, a transactivator of viral genes for lytic replication. Interestingly, ZEB proteins are directly involved in the transcriptional regulation of BZLF1. For example, in B-cell lymphocytes, ZEB1 binds 2 consensus ZEB binding sites in the proximal promoter of the BZLF1 gene to actively repress its transcription. Therefore, inhibition of ZEB1 could be a potential mechanism to break through latency, activate the lytic phase of infection, leading to death of the virally infected malignant cells. Therefore, ZEB expression may have a direct effect on the viral infectious cycle independent of the immune system and, as such, affect the initiation of EBV-related B-cell lymphomas.

**ZEB downstream signaling in epithelial and hematopoietic cells**

ZEB downstream signaling events have mostly been studied in epithelial cells. Differential gene expression analysis upon drug-inducible ZEB1/2 expression resulted in an extensive list of putative ZEB1/2 target genes in the context of EMT. Most of these genes are involved in cellular adhesion, cytoskeletal reorganization, cell polarity, and extracellular matrix composition. Subsequent promoter reporter studies in combination with ChIP identified a common ZEB recognition site consisting of a double E/Z-box in promoters of epithelial marker genes repressed by ZEB1/2. How ZEBs can activate mesenchymal promoters is less understood. Recent work suggested that ZEB1 might act as a direct activator in a complex with other transcription factors, like YAP1. Based on in silico predictions, in combination with in vitro studies using epithelial cancer cell lines, the spectrum of downstream targets of ZEB1/2, at least in the context of EMT, is relatively well established.

In contrast to solid tumor cells, the ZEB transcriptional targets in hematopoietic cells only start to emerge. Gene expression profiling after conditional loss of ZEB2 identified putative effector genes involved in cellular adhesion/homing and chemotaxis (Ccr2, Cxcr3, Cxcr4, Epac, S1PR5, Cxcr3r1, Itgb2, Ccr7, Epac, α4-integrin) as well as lineage-specific cytokines (IL2, IL7R, IL15, IL6, G-CSF). Interestingly, a large proportion of these genes are known targets of E2A. E-proteins are widely expressed bHLH transcription factors that cooperate with tissue/lineage-specific bHLH proteins by forming heterodimers that recognize a single E-box in their target promoter. Id (Inhibitors of DNA binding) proteins counteract E-protein function. They lack a basic DNA-binding domain and tether E-proteins and other bHLH proteins away from E-boxes by forming heterodimers thereby preventing transcriptional E2A activation. Both E-proteins and Id proteins have been shown to play essential roles in hematopoiesis, lymphocyte development, and lymphoid disease, and have been identified as master regulators of CSC and cancer aggressiveness. Therefore, competition between ZEBs and tissue-specific E-protein heterodimers, that bind similar or overlapping DNA recognition sites, could partially explain the ZEB lineage-specific downstream effects (Fig. 5B). Interesting to note in this context is the similarity in function between ZEBs and LMOs. Indeed, while ZEB proteins directly bind bipartite E-boxes via their amino and carboxyterminal zinc finger domains, LMOs are part of a multiprotein transcription complex that can also bridge 2 distant E-protein heterodimers. Interestingly, these similarities might explain the observed phenotypic similarities between ZEB2 overexpression and LMO2 overexpression in the context of T-ALL, spontaneous immature T-ALL formation associated with gain of self-renewal and stem cell properties with tissue/lineage-specific downstream effects.

We noted that multiple ZEB binding sites in promoters of differentially expressed genes upon ZEB2 knockout in NK cells and CD8+ CTLs partially overlap with T-box recognition sites, suggesting that also ZEBs and T-box binding proteins, such as T-bet/Eomes, could influence each other’s DNA binding capacity and transcriptional activity. Such a cross-competition may explain some of the overlapping or synergistic functions in
different hematopoietic lineages.36–38 (Fig. 5B). More research will be necessary to fully understand the synergetic and competitive interactions between these E- and T-box binding transcription factor families. Also competitive or synergetic functions of ZEB proteins with other transcription factor families needs further investigation. As example, Zeb1 indirectly regulates the expression of α4-integrin by inhibiting the transcriptional activity of c-Myb and Ets individually. However, synergy between c-Myb and Ets can overcome this repression, and highlights the fine balance and complex interactions between ZEB proteins and other essential hematopoietic transcription factors.113

The combination of the above-described oscillating ZEB1/2 expression, the cell-state specific chromatin accessibility, the competition with multiple other transcription factors’ families for DNA binding, and the presence or absence of coactivators and repressors, suggests that the transcriptional regulation of ZEB target genes is highly dynamic and cell type-specific resulting in different phenotypes in the loss- and gain-of-function mouse models affecting various stages of normal and malignant hematopoiesis.

Interestingly, EMT transcription factors of the SNAI family are also expressed during hematopoiesis114 and evidence is accumulating that they are also involved in malignant transformation toward leukemia.115 Further research will be necessary to determine whether they use similar converging/complementary pathways in the context of leukemia, as was previously observed during carcinoma progression.12,17

**Conclusion**

Until recently, ZEB proteins have mainly been studied as EMT inducing transcription factors allowing dissemination of epithelial cancers from the primary tumor site, and gaining stem cell properties and features for therapy resistance. Although EMT is not a hallmark of hematologic malignancies, we here enlisted extensive evidence that ZEBs do also play an important role in initiation and progression of different subtypes of lymphomas and leukemias.

Depending on the lineage of origin of these malignancies, ZEB1/2 can both act as oncogenes or tumor suppressors. In certain T-cell malignancies, ZEB1 can be considered as a tumor suppressor and ZEB2 as an oncogene, whereas this seems to be opposite for B-cell malignancies. Finally, in AML, both TFs can act as oncogenes and/or dependency factors. These often contradictory, synergetic, and/or complementary functions of ZEBs on lymphoma/leukemia initiation, progression, or maintenance may be, at least in part, explained by their very pleiotropic functions (cellular adhesion and mobilization/homing, stem cell properties and therapy resistance, immune regulation) at various stages during hematopoiesis; but may also be influenced by their complex oscillating expression profile, in combination with the presence/absence of cell-context dependent cofactors and downstream targets.

This raises the question if it would be feasible to therapeutically target ZEBs in the context of leukemia. Nevertheless, next to the inherent difficulty of targeting transcription factors, the main concern for therapeutic inhibition or reactivation of ZEB proteins might reside in the possible side effects. Indeed, ZEB1/2 have physiological functions throughout the body, including control of the immune system, where they safeguard development and functioning of different immune cell types. Interfering with this could not only disturb response to pathogens, but could also interrupt tumor immunosurveillance. To prevent these important side effects, specific pathways acting downstream of ZEBs, specific interaction with leukemia specific cofactors or their competition for DNA binding with other hematopoietic transcription factors, could be targeted. In that respect, it is interesting to mention that pleiotropic functions of ZEB proteins seem to utilize different protein domains116 and as such the oncogenic roles may be uncoupled from the tumor suppressor roles. More research will be required to dissect the mode of action of ZEB1/2 both in leukemic cells and nontransformed immune cells, which may open the avenue to less toxic and more specific therapies for hematologic malignancies.
References

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