Published in final edited form as:
Ann N Y Acad Sci. 2012 August ; 1266: 47–54. doi:10.1111/j.1749-6632.2012.06625.x.

Caudal genes in blood development and leukemia

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Abstract

The caudal gene family (in mice and humans Cdx1, Cdx2, and Cdx4) has been studied extensively during early development as regulators of axial elongation and antero-posterior patterning. In the adult, Cdx1 and Cdx2, but not Cdx4, have been intensively studied for their function in intestinal tissue homeostasis and the pathogenesis of gastrointestinal cancers. Involvement in embryonic hematopoiesis was first demonstrated in zebrafish, where *cdx* genes render posterior lateral plate mesoderm competent to respond to genes specifying hematopoietic fate, and compound mutations in *cdx* genes thus result in a bloodless phenotype. Parallel studies performed in zebrafish embryos and murine embryonic stem cells (ESC) delineate conserved pathways between fish and mammals, corroborating a BMP/Wnt-Cdx-Hox axis during blood development that can be employed to augment derivation of blood progenitors from pluripotent stem cells *in vitro*. The molecular regulation of *Cdx* genes appears complex, as more recent data suggest involvement of non-*Hox*–related mechanisms and the existence of auto- and cross-regulatory loops governed by morphogens. Here we will review the role of *Cdx* genes during hematopoietic development by comparing effects in zebrafish and mice and discuss their participation in malignant blood diseases.

Keywords

Cdx; hematopoiesis; leukemia; Hox; blood development

Introduction

The caudal (Cdx) family of DNA binding proteins was originally identified in *Drosophila* but homologues with conserved molecular structure and function have been described in several organisms. The three members of the Cdx family in mice and humans, *Cdx1, Cdx2*, and *Cdx4*, have been intensively studied for their involvement in axial elongation and early anteroposterior patterning via *Hox* gene regulation (reviewed by Young and Deschamps[1]). While *Cdx* single and compound deficient mice exhibit overt defects in vertebrae and limbs, studies from zebrafish and murine embryonic stem cells (ESC) indicate additional *Cdx*-driven patterning effects during the development of other mesoderm derivatives such as blood,[2–4] kidney[5] and cardiac[6] cells. Moreover, *cdx* genes regulate the development of neural (e.g. neural tube and spinal cord) and endodermal tissues (e.g. gut) and play important roles in adult intestinal tissue homeostasis and the pathogenesis of gastrointestinal cancers (reviewed by Guo R. J., E. R. Suh and J. P. Lynch[7]).

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The role of *Cdx* genes in the blood system is less well elucidated. Data from zebrafish models demonstrate that *cdx* genes regulate embryonic hematopoiesis through activation of downstream *hox* genes. While overexpression studies performed with murine ESC confirm these data, corresponding *in vivo* loss-of-function studies in mice are complicated by functional redundancy among the three *Cdx* family members. Consistent with the notion that reactivated developmental pathways can contribute to oncogenesis, emerging data indicate expression and functional roles of *CDX2* in leukemia. In this review, we discuss the role of *Cdx* genes during hematopoietic development and their involvement in malignant blood disease.

Insights from knockout mouse models

During early development, *Cdx* genes follow a similar expression pattern to the developmentally related *Hox* genes, conferring positional identity to developing mesodermal tissues. In mice, expression is detected in the posterior epiblast and the overlying mesoderm at the posterior end of the primitive streak.[1] During their development in the posterior growth zone, anterior trunk tissues are exposed to *Cdx* genes but, as cells move anteriorly, *Cdx* transcripts decay.[8–10] Persistence of *Cdx* in the posterior region of the embryo and expression of more posterior *Hox* genes enable the development of posterior trunk mesoderm and tail tissues. The instructive function of *Cdx* and *Hox* genes strongly varies with the developmental stage. As such, overexpression of *hox* genes in the epiblast alters the contribution of cells to the mesoderm[11] and overexpression at the mesoderm stage profoundly impacts morphogenesis of developing tissues such as vertebrae. However, later overexpression in already formed somites shows no effect.[12]

Cdx mutant mice show posterior body truncations involving the axial skeleton, the neuraxis and caudal uro-rectal structures. The severity of the phenotype depends on the individual *Cdx* gene and, consistent with the notion of redundancy, is more pronounced in compound gene knockouts.[13,14] Studies on triple knockout mice are complicated by the essential role of *Cdx2* during placenta development, resulting in lethality of the $Cdx2^{-/-}$ genotype at 3.5 days post coitum (dpc).[15] More recently, inactivation of *Cdx2* at post-implantation stages by a tamoxifen inducible *Cre*-system confirmed the axial truncation phenotype and the incomplete uro-rectal septation in *Cdx2* deficient animals.[16,17] Next to anterior homeotic shifts of the axial skeleton, polyp-like lesions with proximal endoderm have been described in the coecum of $Cdx2^{+/-}$, suggesting anterior homeotic shifts in the intestinal mucosa.[18] Indeed, *Cdx1* and *Cdx2* are expressed in a second wave starting with day 12.5 p.c. in elements of the developing gut and play important roles not only during gut formation but also in adult tissue homeostasis and carcinogenesis.[19] The murine *Cdx4* gene appears less potent than *Cdx1* and *Cdx2*. Analyses of *Cdx2/Cdx4* compound mutants revealed roles for *Cdx4* during placenta development and confirmed redundant roles with *Cdx2* during axial elongation. However, $Cdx4^{-/-}$ mice are born healthy and appear morphologically normal.[20]

In mice, the first hematopoietic cells arise in the yolk sac around 7.5 dpc, representing the primitive wave of hematopoiesis. The second wave of definitive hematopoiesis follows around 9 dpc from hematopoietic stem cells (HSC), which are formed in the aorto-gonado-mesonephros region and then relocate to other anatomic sites including the yolk sac, the fetal liver and, shortly before birth, the bone marrow as the main site of adult hematopoiesis (reviewed by Lengerke and Daley[21]). Single and compound *Cdx* deficient mice do not present overt hematopoietic phenotypes. However, careful analysis has revealed subtle defects such as reduced numbers of yolk sac-derived erythroid colonies in *Cdx4*-deficient versus wild-type mice.[22] Functional redundancy between individual *Cdx* genes may mask effects in single or double knockout mice and targeted triple knockouts have not yet been analyzed.

The *cdx-hox* axis regulates embryonic hematopoiesis

The first link between *cdx* genes and developmental hematopoiesis was made in zebrafish. Homozygous zebrafish embryos carrying the autosomal recessive mutation *kugelig* (*kgg*), initially identified because of their tail defect were found to die early during development (day 5 to 10 post fertilization) and to exhibit pronounced anemia. In 2003, Davidson *et al.* identified loss of function mutations in the *cdx4* gene as the causative mutation for the hematopoietic phenotype of the *kgg* zebrafish embryo.[2] Zebrafish *cdx4* expression occurs in the early gastrula and becomes restricted to the posterior-most cells during gastrulation and early somitogenesis, preceding the expression of hematopoietic markers.[2] *In vivo* injection of *cdx4* mRNA rescues hematopoiesis in *kgg* mutants and induces a "posteriorized phenotype" with ectopic expression of hematopoietic markers in wildtype fish. During development, hematopoietic cells share common progenitors with endothelial cells and arise from so-called *scl*$^{+}$ hemangioblasts. Up to the 5 somite stage, *cdx4* co-expresses with *scl* in the posterior blood islands, suggesting a role for *cdx4* in regulation of *scl*.[2] However, while in wild-type zebrafish embryos hematopoietic cells in the posterior lateral plate mesoderm were expanded by scl overexpression,[23] no rescue of hematopoiesis occurred in *kgg* embryo injected with *scl*-mRNA, indicating that *cdx4* is not a direct inducer of *scl*, or that other co-factors are needed to compensate for the loss of *cdx4*. Moreover, *cdx4* mutations induce a posterior shift in the boundary between anteriorly localized *scl*$^{+}$ cells giving rise to endothelial cells, and the more posterior population of cells, which display hemangioblastic properties. Thus, *cdx4* disruption inhibits the formation of blood but not endothelial cells.[2]

Several developmental studies demonstrate that *Cdx* genes act as master regulators of *Hox* genes.[13,24,25] Consistently, *kgg* mutants display profound alterations in *hox* expression domains with almost complete absence of intermediate and more posterior *hox* genes such as *hoxb6b* and *hoxa9a*, while ectopic *cdx4* can restore hox expression patterns. Moreover, overexpression of individual target *hox* genes (e.g. *hoxb7a, hoxa9a*) also rescue the formation of *gata1*$^{+}$ hematopoietic cells in *kgg* mutants, corroborating the role of a c*dx-hox* axis during blood development. Notably, disruption of the developmental blood program cannot be achieved by targeted inhibition of single *hox* genes, indicating redundant functions of target *hox* genes during blood development.[2] Redundancy has also been observed between *cdx* genes. In zebrafish, the *cdx4* mutation causes a severe but not complete loss of embryonic blood formation. Additional suppression of *cdx1a* in *kgg* mutants however induces a complete failure to specify blood and enhances the severity of *hox* gene deregulation.[3]

Shared upstream and downstream regulatory pathways

Individual *Cdx* genes display not only high amounts of functional redundancy but also share auto- and cross-regulatory molecular loops through direct binding to promoter sites[26] or via regulation of molecular pathways that can act both up- and downstream (e.g. Wnt signaling).[4,14,27]

Interactions with the retinoic acid pathway

Classically, retinoic acid (RA) has been demonstrated as an upstream regulator of *Cdx1* expression.[28,29] *Cdx1* responsiveness to excess RA has been documented *in vivo* and functional RA-responsive elements have been identified in the *Cdx1* gene.[8,28] In the zebrafish embryo, RA modulates the formation of *gata1*$^{+}$ hematopoietic cells.[30] Interestingly, treatment with RA inhibitors rescues hematopoiesis in *cdx* mutants, suggesting that cdx proteins act by modulating the RA pathway.[30] During kidney development, *cdx*-dependent modulation of expression boundaries of the RA synthesizing and degrading *raldh2* and *cyp26a1* regulate the formation of distal tubule segments.[5] In the posterior

growth zone, concomitant regulation of *cyp26a1* expression restraining RA signaling has been demonstrated to participate in the *Cdx-Hox*–orchestrated trunk-to-tail transition.[14] Furthermore, *cdx* inhibition expands the formation of *tbx5a*$^{+}$ anterior lateral plate cardiogenic mesoderm but effective differentiation of *tbx5a*$^{+}$ cells into *nkx2.5*$^{+}$ cardiac precursor cells requires simultaneous suppression of the retinoic acid pathway, supporting the notion that the *cdx* and RA pathways closely interact during development.[6]

Regulation by Wnt, BMP, and FGF

In murine embryoid bodies, BMP and Wnt are necessary for patterning hematopoietic fate from mesoderm. In detail, activation of BMP signaling induces Wnt3a and the canonical Wnt pathway, thereby activating the *Cdx-Hox* pathway.[4] Consistently, mice engineered for loss of responsive elements in the Wnt effector *Lef* display phenotypic effects resembling *Cdx1*$^{-/-}$ mice[29] and *Wnt3a* hypomorph mutants share phenotype with *Cdx2/4* mutants. On the molecular level, direct interactions between *Lef1* and *Cdx1/4* have been demonstrated,[4,31,32] but more complex molecular mechanisms also take place. As such, Wnt proteins have been recently shown to stimulate phosphorylation of the T cell factor (TCF) family member 3 (TCF3), thereby inducing its dissociation from the *Cdx4* promoter and relieving inhibitory effects on *Cdx4* expression.[33,34] Surprisingly, in *Cdx* mutants, *Cdx* expression can be restored and phenotypic posterior defects corrected by posterior gain of function of *Lef1*,[16] suggesting that Wnt signaling also acts downstream of Cdx proteins. These effects were confirmed by studies in ESC, where overexpression of *Cdx1* and *Cdx4* was shown to induce Wnt3a.[4]

Next to RA and Wnt, the Fgf pathway has been involved in activation of *Cdx* genes during development.[35] More recently, Fgf molecules have also been implicated as downstream targets of *Cdx*. For example Fgf8, next to Wnt3a, T and Cyp26a1, is downregulated in *Cdx2* conditional knockout mice and directly responds to *Cdx2*.[17]

Intersection with other transcriptional pathways regulating developmental hematopoiesis

More recently, the signal transduction protein and nuclear transcription regulator *beta-arrestin 1* has been shown to regulate zebrafish hematopoiesis by binding and sequestration of the suppressive polycomb group recruiter *YY1*. Interestingly, overexpression of *cdx4, hoxa9a*, or *hoxb4a* was able to rescue hematopoiesis in *beta-arrestin 1* suppressed fish, suggesting that *beta-arrestin 1- YY1* effects are mediated by the *cdx-hox* axis.[36] Arrestins are known mediators of several developmental pathways important for developmental hematopoiesis such as Wnt, Hedgehog, Notch, and TGF-β.[37] In detail, beta-arrestin1 has been shown to interact with phosphorylated dishevelled proteins and thereby to modulate LEF-mediated transcriptional activity.[38]

Other data from zebrafish models suggest a linear activation of *cdx4* driven by the TATA-box-binding-protein (TBP)-related factor 3, *trf3* (or *tbp2*) through binding of *mespa*. Inhibition of *trf3* during zebrafish development induces multiple defects, including depletion of *cdx4* and a failure to undergo hematopoiesis, which can be rescued by *mespa* expression. Molecularly, *mespa* is a direct target of *trf3* and itself directly activates *cdx4*. Consistently, ectopic *mespa* can rescue the developmental defects of *trf3* suppressed zebrafish embryo, suggesting an ordered *trf3-mespa-cdx4* molecular axis during zebrafish blood development.[39] In mice, the *Mesp1* gene has been reported as one of the earliest markers of cardiac development,[40] but its involvement in hematopoiesis remains unclear. Interestingly, augmentation of *Mesp1* levels can be achieved in differentiating mouse ESC by supplementation with high-dose BMP4, a condition that is also associated with enhanced blood formation (Grauer and Lengerke, unpublished). The molecular interactions between

trf3 and classical morphogen pathways reported to induce *cdx* expression (Wnt, RA, and Fgf) are to our knowledge largely unknown.

To identify additional pathways interacting with *cdx4* during primitive hematopoiesis, Paik *et al.* conducted a chemical screen for compounds that increase *gata1* expression in *cdx4* mutant zebrafish.[41] Only two of 2640 compounds performed a rescue, both belonging to the psoralen family and showing anteroposterior patterning effects similar to DEAB, a known inhibitor of Raldh enzymes. Further analyses are required to discern the molecular mechanisms by which psoralens impact embryonic hematopoiesis, and whether they act downstream or parallel to the *cdx4-hox* pathway.

Cdx proteins direct blood development from pluripotent stem cells

In vitro differentiating pluripotent stem cells (PSC) model early stages of development[21,42] and insights from developmental models can be used to modulate their efficient differentiation into tissues of interest. After exit from the undifferentiated stage, PSC initiate gastrulation and form primitive-streak like cells. During this process, AP-patterning can be imposed by exogenous supplementation with morphogens. For example, BMP4 can stimulate the generation of posterior mesoderm cells and afterwards direct their differentiation into blood cells by activating a Wnt-Cdx-Hox pathway.[4] Accordingly, *Cdx* genes are expressed in waves, peaking during the developmental window of hemangioblast specification in murine and human differentiating PSC.[43] In detail, BMP4 has been shown to induce Wnt3a and activate the *Cdx-Hox* pathway through the Wnt effector molecule Lef1.[4] Overexpression of *Cdx1* or *Cdx4* fully rescues hematopoiesis in the presence of BMP and Wnt inhibition, while overexpression of *Hoxa9* exhibits a partial rescue.[4] Taken together, these data indicate the conserved functions of the BMP-Wnt-Cdx-Hox pathway during blood formation in vertebrates, from zebrafish to mammals.[4]

Single *Cdx* gene deficient mice present surprisingly modest hematopoietic phenotypes. Since overexpression studies indicate redundancy among Cdx genes, loss-of-function studies have been performed on not only single but also on compound *Cdx*-deficient ESC. As expected, *in vitro* differentiation of $Cdx4^{-/-}$, $Cdx1^{-/-}$ and $Cdx2^{-/-}$ ESC reveal only subtle reductions in numbers of blood progenitors obtained in colony forming assays, primarily involving erythroid and mixed progenitor colonies, and a slight decrease in hematopoietic expansion in OP9-coculture assays. However, more profound suppression of hematopoiesis is achieved by knockdown of *Cdx1* and/or *Cdx2* by RNA interference in the background of $Cdx4^{-/-}$ ESC, culminating in an almost bloodless phenotype in $Cdx4^{-/-}$ cells treated with both *Cdx2* and *Cdx1* inhibitory RNAs.[22] Molecularly, these changes are associated with decreased levels of posterior *HoxA* cluster genes. Confirming the notion of *Cdx* gene redundancy, ectopic *Cdx4* can compensate not only for itself but also for *Cdx* compound deficiency. Notably, chimera studies performed with $Cdx2^{-/-}$ and wildtype cells show that the *Cdx* effect is cell autonomous, since no rescue is observed in $Cdx2^{-/-}$ cells exposed to a wild-type environment.[22]

To further dissect individual and redundant effects of *Cdx* family members, doxycycline-inducible *Cdx1, Cdx2*, and *Cdx4* murine ESC have been analyzed in parallel. As previously reported, overexpression of *Cdx1* and *Cdx4* during their endogenous expression window strongly enhances the generation of hematopoietic progenitor cells. [4,44,45] Moreover, transient *Cdx4* overexpression enhances lymphoid engraftment from transplanted ESC-derived hematopoietic progenitors, suggesting inductive patterning effects on definitive hematopoiesis and the generation of hematopoietic stem cells.[22] Interestingly, induction of *Cdx2* during embryoid body development shows suppressive effects on hematopoiesis, possibly mediated by induction of additional (anterior) *Hox* genes.[44] Differential impacts of *Cdx1, Cdx2*, and *Cdx4* are also detected in pre-formed $CD41^{+}$ $ckit^{+}$ blood progenitors

isolated from embryoid bodies. All three *Cdx* genes strongly regulate the hematopoietic potential of $CD41^+$ $c\text{-}kit^+$ cells. However, while *Cdx4* expand hematopoietic colonies, *Cdx1* and *Cdx2* have inhibitory effects, possibly by inhibiting differentiation. Notably, ectopic *Cdx* is unable to re-specify $CD41^-$ cells to hematopoietic fate.[44]

Together, these data demonstrate important roles for Cdx proteins in the specification of hematopoietic progenitor cells. The co-existence of both redundant and non-redundant Cdx-effects, which has been documented also in the gut,[46] may explain the differential impact of the three Cdx genes on adult hematopoiesis and leukemia, which will be discussed below.

Cdx genes in adult hematopoiesis and hematopoietic malignancies

Several genes required during hematopoietic development, such as *Scl/Tal-1* and *AML/Runx1*, have also been shown to regulate adult blood cell homeostasis and malignant transformation.[47] The effect of the three murine *Cdx* genes on $CD41^+c\text{-}kit^+$ ESC derived blood progenitor cells strongly suggests an impact on hematopoietic cell biology beyond early developmental specification. However, in adult whole bone marrow samples of mice and humans, *Cdx1* and *Cdx4* can be detected in only low levels, and *Cdx2* is absent. Lack of *Cdx2* expression in healthy blood cells has been confirmed in analyses of sorted human $CD34^+$ stem and progenitor cells, $CD19^+$ B^- and $CD3^+$ T cells and in murine hematopoietic stem cells (HSC, Lin^- $Sca1^+$ $c\text{-}kit^+$), common myeloid progenitors (CMP), granulocyte-macrophage progenitors (GMP), and megakaryocyte-erythroid progenitors (MEP). Interestingly, aberrant activation of CDX2 can be observed in most cases of acute myeloid and lymphoid leukemia, suggesting a contribution to oncogenic transformation of blood cells. In support of this hypothesis, retroviral overexpression of *Cdx2* in murine whole bone marrow has been shown to enhance *in vitro* serial replating activity and to robustly induce acute myeloid leukemia in mice.[48,49] Molecularly, induction of downstream *Hox* genes such as *Hoxa10* and *Hoxb8* is observed. Thus, acquisition of aberrant *CDX2* expression has been proposed as the mechanism for the deregulated *Hox* expression observed in several leukemias.[48,49] In support of this hypothesis, deletion of the Cdx2 N-terminal domain abrogated its ability to perturb *Hox* genes as well as its leukemogenic activity in mice.[52] Additionally, expression analysis performed on 115 patients with AML showed a correlation between *CDX2* and *HOX* gene levels.[52]

The mechanism by which *CDX2* is reactivated in leukemia remains unclear. In AML, expression of *CDX2* has been shown to be predominantly monoallelic.[48] Amplifications at the 13q12.3 locus of the human *CDX2* gene have been observed in only 3 out of 170 AML patients, all three belonging to the complex karyotype group and showing high *CDX2* transcripts. Despite its first description in a t(12;13)(p13;q12) positive AML,[53] *CDX2* expression levels analyzed in samples from a cohort of 170 patients with AML showed highest expression associated with t(9;11)(p22;p23) translocations, followed by those with normal karyotype, t(15;17)(q22;q11–21), t(8;21)(q22;22), inv(16)(p13q22) or other chromosomal aberrations and complex karyotype, defined as 3 or more cytogenetic abnormalities in the absence of t(8;21), inv(16), t(15;17), or t(11q23). *CDX2* expression levels correlate with disease burden and response to therapy, suggesting possible use of *CDX2* as a marker of minimal residual disease in AML. *CDX2* expression has also been detected in subgroups of patients with myelodysplasia and chronic myeloproliferative leukemia, where, in a low number of analyzed patients, increased *CDX2* expression was associated with transit into secondary AML and respectively with blast and accelerated phase.[48]

As previously mentioned, the downstream pathways regulated by *CDX2* in leukemia are likely to involve *HOX* genes. However, to our knowledge, no data is available showing that

modulation of human *CDX2* expression levels affect *HOX* gene expression patterns in human leukemic cells. Analyses available on *CDX2* in human AML are limited to the correlative expression study mentioned above, showing association between *CDX2* and *HOX* expression levels, and data from AML cell lines showing reduced *in vitro* growth and colony forming capacity after *CDX2* suppression. No functional or molecular data exists in acute lymphoblastic leukemia (ALL), where *CDX2* expression is also a common event.[54,55] Unlike in AML, recent data in pediatric ALL suggest that *HOXA9* expression correlates with better prognosis ($P = 0.03$, $n = 61$ pediatric ALL samples[56]), while *CDX2* is associated with negative prognosis in adult ALL.[55] Intriguingly, *CDX2* expression was not found to correlate with specific *HOX* expression deregulation in pediatric ALL, which may explain why no associations between *CDX2* expression and MLL rearrangements previously associated with *HOX* deregulation have been yet reported in leukemia.[57,58] Moreover, *CDX2* has been directly implicated in the up-regulation of Bcl-2in t(14;18) positive lymphoid cells,[59] partially by interaction with C/EBP.[60] Furthermore, miR-125b is involved as a *CDX2* target regulating hematopoietic cell differentiation through repression of the core binding factor in hematopoietic malignancies.[61] Finally, as reviewed above, more recent data from developmental studies indicates that major morphogen pathways, which also impact leukemogenesis, are downstream regulators of *Cdx*.

The roles of *Cdx1* and *Cdx4* in adult hematopoiesis and leukemia are less well defined. Expression of *CDX1* and *CDX4* has been noted in subgroups of AML in one report,[62] but could not be confirmed in another.[52] Recent data document low levels of *CDX1* but not of *CDX4* in a subgroup of pediatric ALL samples (Grauer and Lengerke, unpublished). In murine models, *Cdx4* has also been shown to activate *Hox* genes and induce myeloid leukemia.[62,63] However, leukemia occurs with a long latency of almost nine months, suggesting that *Cdx4* by itself is insufficient to drive leukemogenesis.[62] More robust contribution of *Cdx4* to leukemia has been shown in combination with other genetic events such as MLL-AF9[64]and Meis1a.[62] Interestingly, *Cdx4* has also proven to be a downstream target of the leukemogenic *HoxA10*.[65] Confirming these data and the studies on embryonic hematopoiesis, no significant effect on during adult hematopoiesis is observed in single knockout *Cdx4* mice.[64]

In summary, these data suggest that *CDX2* is an important co-regulator of malignant transformation in blood cells, but its function and molecular regulation remain poorly understood. The role of *CDX2* may be different in myeloid versus lymphoid neoplasia and its molecular effectors in leukemia likely include non-*HOX* targets. The role of *CDX1* and *CDX4* in adult blood cell homeostasis remain to be defined, however, low or negative expression levels in human leukemia samples suggest modest roles in leukemogenesis when compared to *CDX2*.

Conclusion

Cdx genes are major regulators of embryonic hematopoiesis in zebrafish and conserved roles have been demonstrated in murine pluripotent stem cells, where ectopic *Cdx* can be used to direct blood specification via activation of *Hox* genes. While functional redundancy among the individual *Cdx* family members and compensation during development make it difficult to discern loss-of-function phenotypes in single gene mutant mice, gene specific effects have been revealed by extensive *in vitro* analysis of differentiating pluripotent stem cells. These may be due to differential activation of *Hox*-gene combinations, or to other downstream targets such as morphogens or miRNAs. Non-*Hox*-related effects may explain the activity of *Cdx2* in some leukemia types, especially in MLL-negative ALL and other leukemia in which the role of *Hox* genes is less prominent.

Acknowledgments

C.L. is supported by grants from Deutsche Krebshilfe (*Max-Eder* program), the Deutsche Forschungsgemeinschaft (*SFB773*), the University of Tübingen (*Fortüne* program) and the Böhringer Ingelheim Foundation (*Exploration Grant* program). G.Q.D. is an investigator of the Howard Hughes Medical Institute and supported by grants from the NIH (R24DK092760, UO1-HL100001, RC4-DK090913, P50HG005550, and spezial funds from the ARRA stimulus package- RC2-HL102815), the Roche Foundation for Anemia Research, Alex's Lemonade Stand, Ellison Medical Foundation, Doris Duke Medical Foundation, and the Harvard Stem Cell Institute. G.Q.D. is an affiliated member of the Broad Institute and a senior scientist of the Manton Center for Orphan Disease Research.

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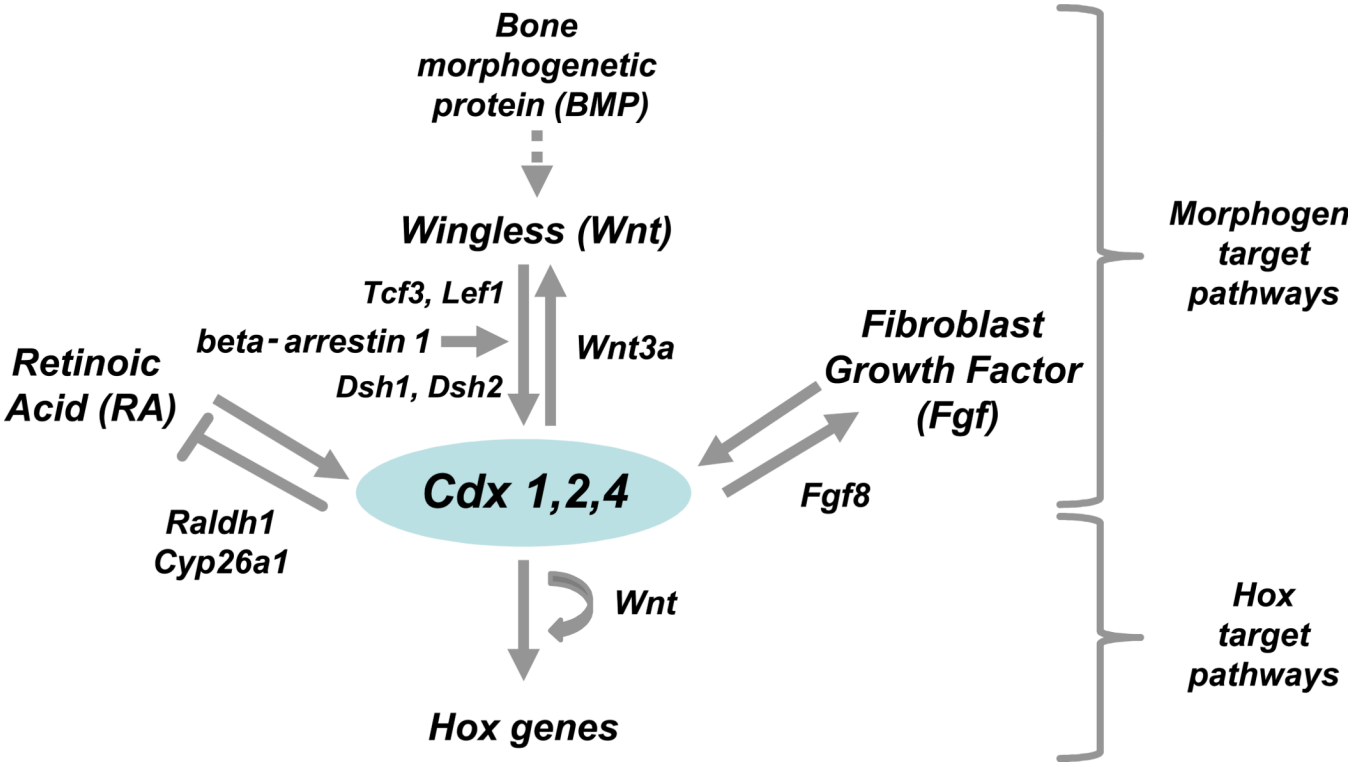

Figure 1.
Schematic view of the molecular interactions between Cdx and morphogen pathways.