Brief report

Aberrant expression of the homeobox gene *CDX2* in pediatric acute lymphoblastic leukemia

Tamara Riedt,[1] Martin Ebinger,[2] Helmut R. Salih,[1] Jürgen Tomiuk,[3] Rupert Handgretinger,[2] Lothar Kanz,[1] Frank Grünebach,[1] and Claudia Lengerke[1]

[1]Department of Hematology/Oncology, University of Tuebingen Medical Center II, Tuebingen; [2]Department of Hematology/Oncology, University of Tuebingen Children's Hospital, Tuebingen; and [3]Division of General Human Genetics, Institute of Human Genetics, University of Tuebingen, Tuebingen, Germany

Members of the caudal (*cdx*) family of homeobox proteins are essential regulators of embryonic blood development in zebrafish. Previously, we reported that the murine homologues (*Cdx1, Cdx2,* and *Cdx4*) affect formation and differentiation of embryonic stem cell (ESC)–derived hematopoietic progenitor cells. Consistent with the notion that embryonic pathways can reactivate during adult oncogenesis, recent studies suggest involvement of *CDX2* in human acute myeloid leukemia (AML). Here we study *CDX2* in healthy and leukemic human lymphoid cells, and show that a majority of leukemic samples display various degrees of aberrant *CDX2* expression. Analysis of a cohort of 37 childhood acute lymphoblastic leukemia (ALL) patients treated in our hospital reveals that high *CDX2* expression levels at diagnosis correlate with persistence of minimal residual disease (MRD) during the course of treatment. Thus, *CDX2* expression levels may serve as a marker for adverse prognosis in pediatric ALL. (Blood. 2009;113:4049-4051)

Introduction

Cdx genes are classically known as regulators of axial elongation and anterior-posterior patterning during early embryogenesis. Recent studies have revealed an unsuspected role during hematopoietic development: in zebrafish embryo, *cdx1a* and *cdx4* redundantly induce blood formation via activation of specific posterior *hox* genes.[1,2] During in vitro differentiation of embryonic stem cells (ESCs), the 3 murine *Cdx* genes (*Cdx1, Cdx2,* and *Cdx4*) also modulate *Hox* gene expression,[3-6] thereby participating in mesodermal patterning to blood fate[4] and regulation of preformed hematopoietic progenitors.[5]

Little is yet known about the role of *CDX* genes in human hematopoiesis. The *ETV-CDX2* fusion gene has been identified in a patient with acute myeloid leukemia (AML) carrying the rare translocation t(12;13)(p13;q12).[7] Recently, aberrant *CDX2* expression was detected in most cases of AML.[8,9] Currently available studies have focused on *CDX2* in AML, possibly because retroviral overexpression of *Cdx2* in murine bone marrow promotes AML.[8] However, during hematopoietic development from ESCs, *Cdx4* overexpression enhances formation of progenitors with lymphoid repopulation capacity,[6] suggesting involvement of *Cdx* genes in lymphopoiesis. Moreover, aberrant expression of potential (*HOX*) target genes has been observed in acute lymphoblastic leukemia (ALL).[10-12] Here we determine *CDX2* expression in healthy and leukemic lymphoid cells, and explore the prognostic impact of *CDX2* transcript quantitation for the clinical course of pediatric ALL by analyzing a cohort of 37 patients.

Methods

Bone marrow (BM) and peripheral blood (PB) samples were collected from healthy donors and patients presenting with leukemia. All patients, or when appropriate their legal guardian, gave their written informed consent form in accordance with the Helsinki protocol, and the study was performed according to the guidelines of the Ethics Committee of the University of Tuebingen, Tuebingen, Germany. In pediatric ALL, we followed a cohort of 37 patients treated in our hospital between 2003 and 2008. Induction therapy was applied according to the ALL-BFM protocol, and minimal residual disease (MRD) diagnosis performed as previously described.[13,14] Patients' characteristics are shown in Table S1, available on the *Blood* website; see the Supplemental Materials link at the top of the online article. Here we describe only briefly the processing of samples that is presented in more detail in Document S1: a LightCycler carousel-based system and LightCycler TaqMan Master chemistry (Roche, Mannheim, Germany) were used for real-time reverse-transcription polymerase chain reaction. Relative *CDX2* expression levels were calculated after normalization to the housekeeping gene *PBGD*. Statistical analysis was performed using the Wilcoxon rank test (SAS system, procedure NPAR1WAY; SAS Institute, Cary, NC) and the Fisher exact test (SAS system, FREQ Procedure).[15] The significance level α equals .04 for all tests.

Results and discussion

Cdx genes regulate hematopoiesis by modulating posterior *Hox* genes during embryonic development.[1-6,16] In adults, *Cdx4* but not *Cdx2* expression was detected in murine bone marrow.[8,17] *CDX2* was not expressed in healthy human BM mononuclear cells (MNCs), isolated $CD34^+$, or myeloid progenitor cells.[8] In our laboratory, analysis of healthy donor BM, PB, and purified B ($CD19^+$), T ($CD3^+$), or natural killer (NK; $CD56^+$) cells showed negative or very low *CDX2* expression (defined as *CDX2/PBGD* [%] $0 <$ ratio < 1; Figure S1A), whereas MNCs collected from patients presenting with AML expressed *CDX2* in 9 of 10 cases, which is line with previously reported results[8] (Table 1A). In

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Table 1. High *CDX2* transcript levels correlate with poor elimination of MRD in pediatric ALL

CDX2 expression	Pediatric ALL, no. of patients		
	MRD point in time 1	MRD point in time 2	
		Positive	Negative
CDX2/PBGD ratio, % 0 ≤ ratio < 4	8	3	5
CDX2/PBGD ratio, % 4 ≤ ratio	9	9	0

Negative/low *CDX2* expressers convert significantly better from positive MRD status at MRD point in time 1 to negative MRD status at MRD point in time 2, in comparison with pediatric ALL patients showing higher initial *CDX2* transcripts (CDX2/PBGD ratio [%] 4 ≤ ratio). (SAS procedure FREG; Fisher exact test, $P = .016$).

contrast, aberrant *CDX2* (defined as *CDX2/PBGD* ratio [%] 1 ≤ ratio) was found in 3 of 7 analyzed lymphoid neoplastic cell lines, with highest levels in NALM-16 cells derived from a patient with pediatric ALL (Figure S1B). Translation into aberrant CDX2 protein was confirmed (Figure S1C). Next, we determined *CDX2* transcripts in primary samples collected from patients with pediatric ALL. *CDX2* expression was detected in all common ALL (c-ALL), and the majority of analyzed T-cell ALL (T-ALL) samples (78%). Overall, only 6 of 37 pediatric ALL patients (16%) showed negative or very low *CDX2* expression comparable with levels in healthy donors. *CDX2* expression was similarly high in 3 adult ALL samples (Figure 1). Two samples from pediatric ALL patients revealed extremely high expression (*CDX2/PBGD* ratio 288.16% and 107.46%, respectively) and were thus considered outliers and not included in the statistical analysis. Among the remaining 35 pediatric ALL patients, mean expression was 7.59 (expression range, 0 to 32.88). Patients were divided into 4 groups: negative/low expressers (*CDX2/PBGD* ratio [%] 0 ≤ ratio < 4), low-intermediate expressers (4 ≤ ratio < 8), high-intermediate expressers (8 ≤ ratio < 12), and high expressers (12 ≤ ratio). The negative/low expressers group comprised all samples from healthy donors (Figure 1). Even in this group, mean *CDX2/PBGD* ratio (%) in pediatric ALL patients (1.74 ± 1.25) was significantly higher than in healthy controls (0.375 ± 0.41) (Wilcoxon rank test, 1-sided, $P = .009$), whereas no significant differences were noted among c-ALL (1.76 ± 1.28), T-ALL (1.83 ± 1.35), and AML (1.45 ± 0.94) (Figure 1). To explore whether *CDX2* was specific to acute leukemia or also a feature of other lymphatic neoplasias, samples from 11 patients with chronic lymphocytic leukemia (CLL) were included in the study. More frequently than ALL patients, CLL patients belonged to the negative/low expressers group (72% vs 43%; Figure 1). Our data suggest that high *CDX2* levels may correlate with more aggressive disease. However, analysis of more CLL samples is needed for conclusive comparisons.

Pediatric ALL is the most common malignancy of childhood, and is treated with chemotherapy alone or in combination with radiation therapy and, in selected cases, with allogeneic stem cell transplantation. Long-term cure and survival rates have significantly improved by adapting therapy intensity to prognostic factors associated with disease aggressiveness and recurrence. Currently, the most important prognostic parameter represents the early in vivo evaluation of therapy response by measuring leukemic cell persistence after induction therapy (minimal residual disease, MRD).[13,14,18] We questioned whether *CDX2* expression at diagnosis could predict course of the disease by analyzing association with MRD[13,14] measured at the point in time 1 (day 33 after start of chemotherapy—first regeneration during induction therapy) and the point in time 2 (2 weeks after completion of induction around day 80). MRD assessment was performed in 30 of 37 patients. At the point in time 1, 17 of 30 analyzed patients were MRD positive. At the point in time 2, 5 of these 17 patients became MRD negative, whereas in 12 patients leukemic cells persisted. There was no MRD-positive patient at the point in time 2 who was previously MRD negative. MRD positivity at the point in time 1 was seen in a comparable number of *CDX2* negative/low expressers (n = 8) and patients with initial *CDX2/PBGD* ratio (%) 4 ≤ ratio (n = 9). However, conversion rates between MRD points in time 1 and 2 were significantly better for negative/low *CDX2* expressers, compared with patients

CDX2 expression levels; CDX2/PBGD ratio (%)

	Negative/ Low ratio < 4	Low intermed. 4 ≤ ratio < 8	High intermed. 8 ≤ ratio < 12	High 12 ≤ ratio	*P-value**
Healthy donors (n=8)	0.375±0.41 *(n=8)*	- *(n=0)*	- *(n=0)*	- *(n=0)*	
ALL (pediatric and adult ALL, n=40)	1.79±1.23* *(n=17)*	5.56±0.98 *(n=8)*	10.06±1.17 *(n=6)*	25.88±5.51 *(n=7)*	*0.007*
Pediatric ALL (n=37)	1.74±1.25* *(n= 16)*	5.56±0.98 *(n=8)*	10.06±1.17 *(n=6)*	26.62±4.18 *(n=5)*	*0.009*
Pediatric c-ALL (n=26)	1.76±1.28* *(n=8)*	5.56±0.98 *(n=8)*	9.92±1.25 *(n=5)*	26.5±4.81 *(n=4)*	*0.010*
Pediatric T-ALL (n=9)	1.83±1.35* *(n=7)*	- *(n=0)*	- *(n=0)*	27.11 *(n=1)*	*0.054*
Adult ALL (n=3)	2.74 *(n=1)*	- *(n=0)*	- *(n=0)*	24.03±10.13 *(n=2)*	-
Adult B-CLL (n=11)	1.47±1.26* *(n=8)*	16.78 *(n=1)*	- *(n=0)*	15.68±2.69 *(n=2)*	*0.042*
Adult AML (n=10)	1.45±0.94* *(n=6)*	4.9±0.56 *(n=2)*	- *(n=0)*	19.43±3.93 *(n=2)*	*0.022*

Figure 1. CDX2 expression in leukemic and healthy control primary BM and PB samples. Patients presenting with leukemia show significantly higher *CDX2* expression than healthy controls. *CDX2/PBGD* ratio (%) was analyzed for leukemia and healthy donor BM or PB samples. Healthy donors and leukemia patients were grouped according to their CDX2/PBGD ratio (%): low expressers (0 ≤ ratio < 4); low-intermediate expressers (4 ≤ ratio < 8); high-intermediate expressers (8 ≤ ratio < 12); and high expressers (12 ≤ ratio). Numbers represent mean *CDX2/PBGD* ratio (%) ± SD in each subgroup consisting of n patients. *Significant differences between low expressers from leukemia groups compared with the healthy controls, as determined by Wilcoxon 2-sample, 1-sided test, SAS system, NPAR1WAY procedure. ALL indicates acute lymphoblastic leukemia; c-ALL, common acute lymphoblastic leukemia; T-ALL, T-cell acute lymphoblastic leukemia; B-CLL, B-cell chronic lymphocytic leukemia; and AML, acute myeloid leukemia.

with higher initial *CDX2* expression (conversion to MRD negativity 62.5% vs 0%, $P = .016$, Fisher exact test; Table 1). Our data suggest that *CDX2* expression levels at disease manifestation may be used to anticipate therapy response in patients showing positive MRD at day 33. At this point, it remains speculative whether leukemic blasts with low *CDX2* expression levels may have a different biology than blasts with high *CDX2* expression, and whether the latter are specifically resistant to the chemotherapy regimen applied between MRD points in time 1 and 2 (consisting mainly of cyclophosphamide and low-dose cytarabine), rather than to the intensive induction regimen applied prior to day 33 (consisting of high-dose steroids, daunorubicine, asparaginase, and vincristine). Analysis of larger cohorts of patients and replication in independent samples from different populations should further support the validity of this association and determine whether patients with higher *CDX2* expression would benefit from alterations of current treatment protocols.

In summary, our study demonstrates that aberrant *CDX2* expression is not a specific feature of myeloid neoplasia, but also extends to lymphoid neoplasia. *CDX2* expression at diagnosis can be correlated with the clinical course of pediatric ALL and may be useful as a risk stratification marker in patients with pediatric ALL.

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Authorship

Contribution: T.R., M.E., and F.G. designed and performed experiments, and analyzed results; H.R.S. contributed critical samples; J.T. performed statistical analysis; L.K. designed the research and reviewed the paper; R.H. designed the research; C.L. designed the research, analyzed results, and composed this paper; and all authors contributed to editing of the paper.

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Correspondence: Claudia Lengerke, Department of Hematology/Oncology, University of Tuebingen Medical Center II, Otfried-Mueller Strasse 10, 72076 Tuebingen, Germany; e-mail: claudia.lengerke@med.uni-tuebingen.de.

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