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The Cardiac Homeobox Gene *NKX2-5* Is Deregulated by Juxtaposition with *BCL11B* in Pediatric T-ALL Cell Lines via a Novel t(5;14)(q35.1;q32.2)[1]

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ABSTRACT

A cryptic chromosome rearrangement, t(5;14)(q35.1;q32.2), recently identified in pediatric acute lymphoblastic leukemia (ALL), targets activation of *TLX3* at 5q35.1 by juxtaposition with a region downstream of *BCL11B* at 14q32.2. We describe a novel variant t(5;14) whereby *NKX2–5*, a related (NK-like family) homeobox gene located ~2 Mb telomeric of *TLX3*, juxtaposes *BCL11B* in a subset of T-cell ALL cell lines. In this t(5;14) variant, *NKX2–5* is expressed instead of *TLX3* at both RNA and protein levels. Subsequent expression screening failed to detect involvement of additional NK-like genes in T-cell ALL cells. Our data pinpoint a regulatory region far downstream of *BCL11B* effecting ectopic homeobox gene activation. This study also identifies *in vitro* models for both t(5;14) variants and raises questions about diagnostic fluorescence *in situ* hybridization/reverse transcription-PCR screening in ALL.

INTRODUCTION

Recurrent but cryptic chromosome changes in leukemia are increasingly being identified by FISH.[3] A recent example is t(5;14)(q35.1;q32.2) present in ~20% of children with T-ALL (1–3). Formation of t(5;14) is believed to target deregulation of a homeodomain gene *TLX3* (also called *HOX11L2*) at 5q35.1 via its juxtaposition with the distal region of a krueppel-like zinc-finger transcription factor *BCL11B* (also called *CTIP2*) at 14q32.2, which is preferentially expressed in T cells (1, 4, 5). *TLX3* belongs to the NK-like family of homeobox genes (6), which also includes *TLX1* located at 10q24 and is involved in at least two leukemic translocations in T-ALL, t(7;10)(q35;q24) and t(10;14)(q24;q11.2), effecting its juxtaposition with *TRB@* and *TRD@*, respectively. Extending this paradigm, Hansen-Hagge *et al.* (7) have described two adult ALL patients with t(5;14)(q35;q11.2) with breakpoints upstream of *TLX3* and within *TRD@*. Although the leukemic involvement of homeobox genes is known, the role of *BCL11B* remains unclear, although its close homologue, *BCL11A*, expressed in B cells and derived malignancies, is cast as a dominant oncogene (4). While screening T-ALL cell lines by FISH, we found examples with t(5;14) in which the 5q35 breakpoints outlay *TLX3*. We describe the characterization of these cell lines to reveal an alternative homeobox gene target of t(5;14) showing that the downstream region of *BCL11B* is capable of activating at least two NK-like homeobox genes.

MATERIALS AND METHODS

Cell Lines. CCRF-CEM was established in 1964 from a 3-year-old female with relapsing T-ALL. PEER was established in 1977 from a 4-year-old female at second relapse of T-ALL. CCRF-CEM and PEER exhibit immature imunophenotypes, being classed T-III cortical/T-cell receptor $\alpha\beta+$ and T-IV/T-cell receptor $\gamma\delta+$, respectively. Details of these and other cell lines used in this study and obtained from the DSMZ[4] are given in Drexler (8). DNA profiling has shown that the purportedly independent cell lines, MKB-1 and BE-13, are early passage divergent subclones of CCRF-CEM and PEER, respectively (9).

Cytogenetic Analysis. Harvesting, slide preparation, trypsin G-banding (GTG), and FISH were performed as described (10). BAC clones were obtained from BAC/PAC Resources (Oakland, CA), RZPD (Berlin, Germany), The Sanger Centre (Cambridge, England), J. Flint (Oxford, England), J.W.G. Janssen (Heidelberg, Germany), and R. Siebert (Kiel, Germany). Clone DNA was prepared using commercial kits (Qiagen, Hilden, Germany) and labeled by nick translation (Invitrogen, Karlsruhe, Germany). FISH images were analyzed using commercial software (Applied Imaging, Newcastle, United Kingdom).

Analysis of Gene Expression. For RT-PCR, cDNA was synthesized from 5 μg of total RNA extracted from 2×10^6 cells with TRIzol (Invitrogen) by random priming in 20 μl using Superscript II (Invitrogen). Three microliters of cDNA template were amplified by PCR in 25 μl and checked using *UBTF* primers (Table 1). Incubation steps were as follows: 1 min/94°C, 30 s/55°C (for deviations, see Table 1) and 3 min/72°C for 35 cycles using a thermal cycler (Perkin-Elmer, Wiesbaden, Germany). PCR products were analyzed on 1.5% agarose gels and cloned in pGEM-Teasy (Promega, Madison, WI) for sequencing (MWG Biotech, Ebersberg, Germany). For Northern analysis, 10 μg of total RNA were loaded onto agarose gel, transferred onto nylon membranes (Roche, Mannheim, Germany), and UV cross-linked (Stratagene, Heidelberg, Germany). Full-length cDNA probes of hamster β-actin (~1200 bp) and *NKX2–5*, generated by RT-PCR (Table 1), were random primed labeled with ^{32}P (Stratagene). Detection was performed by phosphoimaging (Storm, Krefeld, Germany). For Western analysis, 4×10^6 cells were lysed with 50 μl of RIPA-buffer, 1 μl of aprotinin (1 mg/ml), 5 μl of phenylmethylsulfonyl fluoride (20 ng/ml), and 50 μl of 2 $\times$ SDS buffer. Lysates (20 μl) were loaded onto 15% SDS-PAGE gels (Bio-Rad, Munich, Germany) and blotted onto nitrocellulose membranes (Schleicher Schuell, Dassel, Germany) by the semi-dry method. Detection of α-NKX2–5 (H-114) and α-TLX1 (C-18; Santa Cruz Biotechnology, Heidelberg, Germany) used a Western lighting kit (Perkin-Elmer). Protein loading equivalence was checked using Ponceau dye.

RESULTS

FISH Screening. We screened 22 T-ALL cell lines for t(5;14) by FISH and for *TLX3* expression by RT-PCR. FISH with flanking clones at 5q35 (centromeric of *TLX3* and a 5q subtelomeric probe) and 14q32 (centromeric of *TCL1* and a 14q subtelomeric probe) yielded the following positives, CCRF-CEM/MKB-1 and PEER/BE-13. Conventional cytogenetic analysis and chromosome painting revealed the following karyotypes: CCRF-CEM - 46,XX,t(8;9)(p11;p24),der (5)t(5;14)(q35.1;q32.2),der (9)del (9)(p24) del (9)(q11q13–21),ins(14;5)(q32.2;q35.1q35.1),+20; PEER - 46,XX,der (4)dupins (4;4)(p11;q21q25),der (5) del (5)(q22q31)t(5;14)(q35.1;q32.2),del (6)(q13q22),del (9)(p11p22),del (9)(q22),ins (14, 5)(q32.2;q35.1q35.1). Karyotypic images are depicted in Figs. 1, *A* and *B*. As shown previously for HPB-ALL (5), CCRF-CEM, PEER, MKB-1, and BE-13 all carry the cryptic t(5;14), undetectable by chromosome painting (Fig. 1*C*), involving breakpoints at 5q35.1 and 14q32.2 Investigation with BAC/PAC clones (Fig. 1, *D* and *E*) showed that although breakpoints at 14q32.2 matched those described previously, those at 5q35.1 in PEER and CCRF-

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[3] The abbreviations used are: FISH, fluorescence in situ hybridization; ALL, acute lymphoblastic leukemia; NK, natural killer; BAC, bacterial artificial chromosome; RT-PCR, reverse transcription-PCR; T-ALL, T-cell ALL; *UBTF*, upstream binding transcription factor.

[4] Internet address: http://www.dsmz.de.

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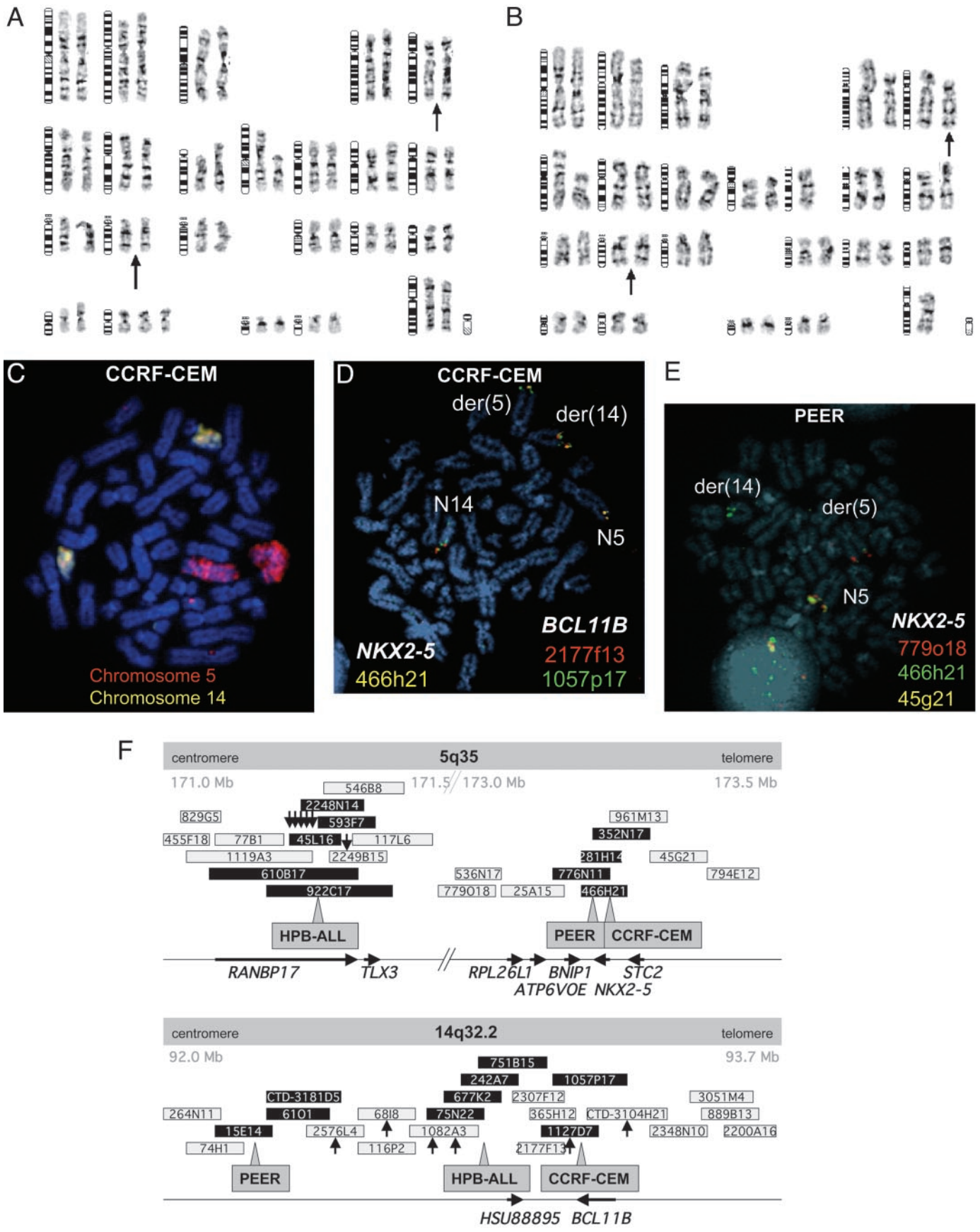

Fig. 1. FISH analysis of t(5;14)(q35.1;q32.2). *A* and *B*, G-banded karyotypes of CCRF-CEM and PEER cell lines, respectively. Both partners of the cryptic t(5;14) are indistinguishable from their normal homologues (*arrows*). However, PEER carries a simultaneous deletion of q22-q31 on the der (5)t(5;14). Both cell lines carry additional secondary changes. FISH image (*C*) shows the results of hybridizing a CCRF-CEM metaphase with painting probes for chromosomes 5 (*red*) and 14 (*yellow*). Note the absence of any visible

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Table 1 *Genes analyzed by RT-PCR*

Gene	Acc. no.	Primer direction[a]	Primer sequence (5′-3′)	Annealing temp. (°C)	Product (bp)	Remark	*Drosophila* Orthologue
BCL11B	XM017303	F	GCCAGTGTCAAATGAACTTCC				
		R	CATGTTGTGCAAATGTAGCTGG	55	292	Variant 2	
		R2	CTGACACTGGCATCCAAAGG	55	458	Variant 1	
BNIP1	NM001205	F	CAGTTGCGTCACAGAATACAG				
		R	TCAGTGATGGTACTGGATGTC	55	262		
TLX1	XM046733	F	GCGTCAACAACCTCACTGGCC				*93 Bal*
		R	GTGGAAGCGCTTCTCCAGCTC	55	170		
TLX2	NM001534	F	CTTCCTGCGCCAGAAGTACC				*93 Bal*
		R	AGAGCGACGAGTTGTGCAGG	55	222		
TLX3	XM003705	F	GCGCATCGGCCACCCCTACCAGA				*93 Bal*
		R	CCGCTCCGCCTCCCGCTCCTC	55	242		
LBX1	X90828	F	CGCCAGCAAGACGTTTAAGG				*lbe, 1bl*
		R	AGCTGCTGCGCGATTTGGTC	55	213		
LBX2	AC005041	F	GCGCTGGAGGAGCTGACTAG				*1be, 1bl*
		R	CGCGCATCTCCTCCACATCG	60	307		
NKX2-1	NM003317	F	AGGACACCATGAGGAACAGC				NK2 (*vnd*)
		R	CCGACAGGTACTTCTGTTGC	55	261		
NKX2-2	NM002509	F	GGACATCTTAGACCTGCCGG				NK2 (*vnd*)
		R	TTGGAGCTTGAGTCCTGAGG	55	266		
NKX2-3	XM089498	F	CTATGTCCACACGGTCCTGC				NK4 (*tin*)
		R	GAGTGGATGTGAGCTTCAGGC	55	297		
NKX2-4	AF202037	F	GCAACATGGGCGAGCTGCC				NK2 (*vnd*)
		R	TTGAAGCGCCGCTCCAGCTC	55	270		
NKX2-5	NM004387	F	TCTATCCACGTGCCTACAGC				NK4 (*tin*)
		R	TGGACGTGAGTTTCAGCACG	55	264		
		XF	CCGAATTCTGCCGCCCACCTGGCGCTGTG				
		XR	CCAAGCTTCCCTACCAGGCTCGGATACCAT		1021	Full length	
		F2	GAGCCAGCCTGACTTTCTAC	55	298	Splicing	
		F3	TAGGTCAAGCCGCTCTTACC	55	256	Splicing	
		1aF	TGCTCAGACAGCCCAGAAAGCAG				
		1aR	GAACATGGTGGCAGCGCCAGTC	55	353	Exon 1a	
NKX2-6	XM070619	F	AAGCTGGATGGTTCGGAGCC				NK4 (*tin*)
		R	GTACCGCTGCTGCTTGAAGC	55	273		
NKX2-8	NM014360	F	TCTGGACGCCTGAGCTTCAC				NK2 (*vnd*)
		R	GCGCCTTGGAGAATAGCACC	55	274		
NKX3-1	AF247704	F	GTGCATTCAGGCCAAGGCGG				NK3 (*bab*)
		R	CGCTTCTGCGGCTGCTTAGG	57	291		
NKX3-2	NM001189	F	ATCCTTGAGCCTCGGCCAGC				NK3 (*bab*)
		R	GCTCCAGCTCGAAGACCTGC	57	305		
RANBP17	NM022897	F	AACCAGTGGTCAGTATCCAG				
		R	CTGCATGGTTCAGTGTTGCC	55	256		
STC2	NM_003714	F	TACCAAGAACCATGTGTGCC				
		R	TGATGAATGACTTGCCCTGG	55	323		
UBTF	X53390	F	CTGGAATGCATGAAGAACAACC				
		R	TCTTGGTTAGGTCCAGGTTGC	55	342		

[a] F, Forward primer; R, reverse primer.

CEM (both studied in detail), as well as in MKB-1 and BE-13, lay ~2 Mb telomeric to those reported previously at 3′-*TLX3* (Refs. 1 and 5; Fig. 1*F*). Neither did these cell lines express *TLX3* (Fig. 2; Table 2). On the basis of the configurations of the BAC FISH signals, breakpoints at 5q35.1 in both cell lines were assigned within a short overlapping trios of BACs, 466H21/281H14/352N17, in CCRF-CEM, and 776N11/466H21/281H14, in PEER (Fig. 1, *D–F*). Thus ~200 kb of DNA in CCRF-CEM and 150 kb DNA in PEER were inserted at 14q32.2. Reference to the Ensembl browser (April 2003 freeze) showed that the overlapping region shared by these insertions bore a single expressed gene, *NKX2–5*. FISH analysis placed 14q32.2 breakpoints between the following pairs of overlapping BAC clones: 1127D7 and 1057P17 in CCRF-CEM (centered at 93.182 Mb); and within 15E14, between 74H1 and 61O1 in PEER (centered at 92.289 Mb). Thus, the 14q32.2 breakpoints in CCRF-CEM and PEER lay 0.37 and 1.27 Mb downstream of *BCL11B*, respectively, tightly flanking those described hitherto in patients and HPB-ALL (Fig. 1*F*).

Mutational Analysis and Gene Expression at 5q35.1. We analyzed genes flanking the breakpoint regions of chromosomes 5 (*BNIP1, NKX2–5,* and *STC2)* and 14 (*VRK1, HERV/HSU88895,* and *BCL11B)* by RT-PCR. *BNIP1* interacts with E1B and BCL2 proteins to control apoptosis (11), *NKX2–5* encodes a homeobox gene expressed in heart and spleen (12), and *STC2* codes for a glycoprotein hormone regulating Na-phosphate metabolism in the kidney (13). *STC2* expression was detected HELA (cervix ca.), but neither in CCRF-CEM nor PEER nor in any other T-ALL cell line analyzed (data not shown). In contrast, *BNIP1* was expressed in all cell lines analyzed (8 of 8), regardless whether t(5;14) was present. Crucially, among hematopoietic cells, expression of *NKX2–5* was restricted to CCRF-CEM, PEER (Fig. 2*A*), and their respective subclones MKB-1

rearrangement. *D* and *E*, analysis of the breakpoints at *NKX2–5* and 3′-*BCL11B*. In CCRF-CEM (*D*), split BAC FISH signals show 5q35 breakpoints at *NKX2–5* (*yellow*), whereas the presence of green signal only on the der (*5*) shows the 14q32 breakpoint occurred between 2177F13 and 1057P17. In PEER (*E*), the presence of signal on the der (*14*) from 466H21 (*green*) without signals from flanking centromeric (*red*) or telomeric (*yellow*) clones documents the mini-insertion of *NKX2–5* material. Note the weaker green signal on the der (*5*) attributable to the centromeric breakpoint within 466H21. Metaphase spreads were hybridized with contrasting BAC/PAC clones labeled with Spectrum Red-dUTP, Spectrum Green-dUTP, or Cy3-dUTP. Chromosomes were counterstained with DAPI, and images were captured and analyzed using special software (Applied Imaging). A diagrammatic representation of BAC/PAC clones referred to in this study is shown in *F*. The *top* and *bottom* parts depict 5q35 and 14q32 breakpoints in CCRF-CEM, PEER, and HPB-ALL cell lines (*arrowheads*), relative to those described by Bernard *et al.* (1; *arrows*) and to their putative gene targets. Clones straddling (split-signal), or immediately flanking breakpoints, are shaded darkly. Breakpoints were inferred by mapping to overlapping split-signal clones. Contrast the clustering of 5q35 breakpoints at 5′-*NKX2–5* and 5′-*TLX3* with the scattering of 14q32 breakpoints over 3′-*BCL11B*. The cytogenetic data pinpoint the putative enhancer to the far downstream region of *BCL11B*, centromeric of the breakpoint in PEER.

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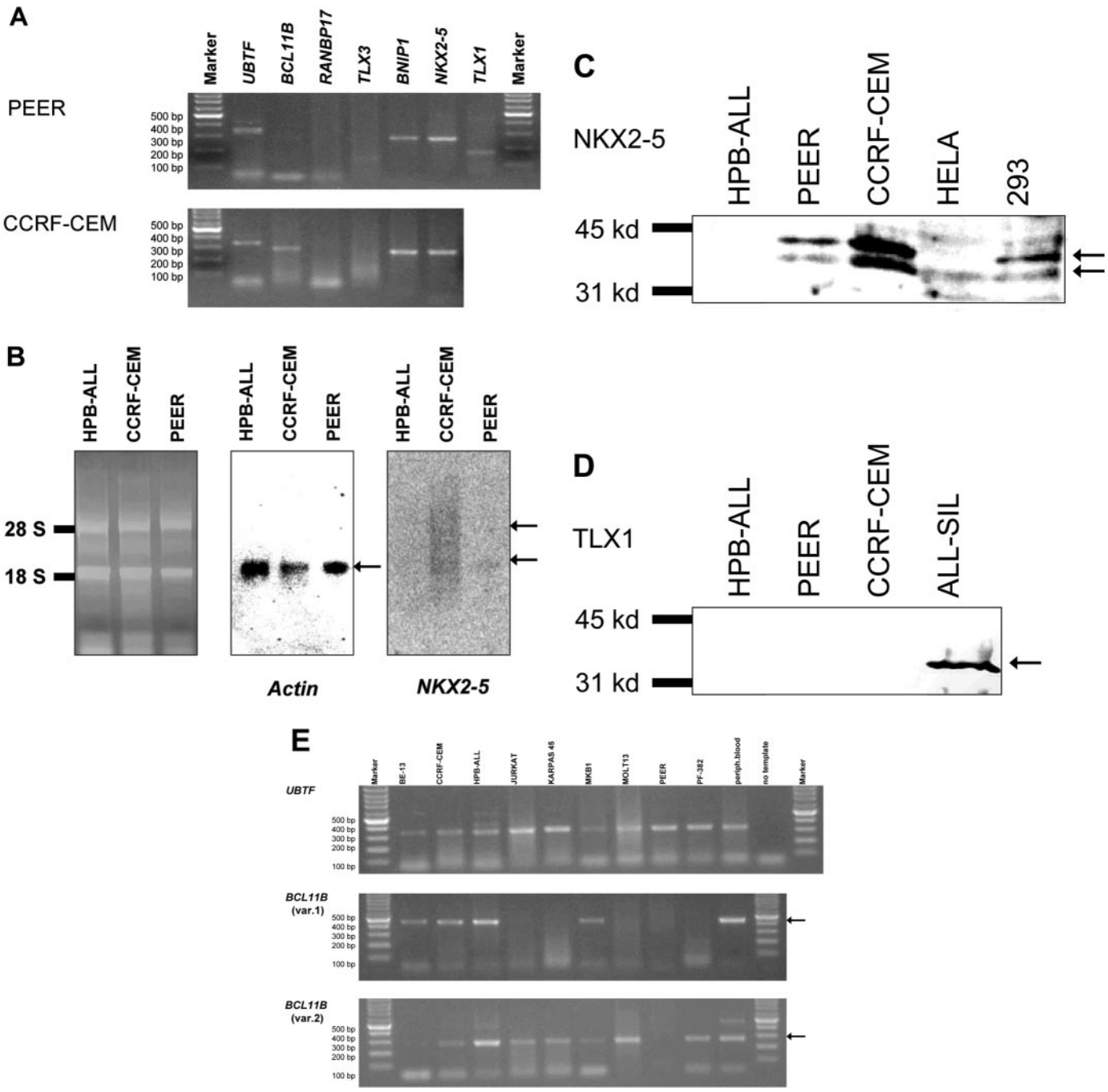

Fig. 2. Gene expression analysis. *A*, the results of RT-PCR analysis. PEER showed expression of *BNIP1, NKX2–5,* and *TLX1,* whereas *BCL11B, RANBP17,* and *TLX3* were negative. CCRF-CEM was positive for *BCL11B, BNIP1,* and *NKX2–5* but negative for *RANBP17* and *TLX3*. *UBTF* expression served as a cDNA control. The results of Northern analysis of *NKX2–5* are shown in *B.* At least two signals (*arrows*) at ~1.8 and 3.6 kb were visible using the *NKX2–5* probe. Total RNA from HPB-ALL, CCRF-CEM, and PEER was separated on agarose gel (*left*). The blot was hybridized with ^{32}P-labeled probes for actin (*middle*) and *NKX2–5* (*right*). The image in *C* illustrates the detection of *NKX2–5* by Western blot. Two bands at M_r ~35,000 and 37,000 (*arrows*) are visible in PEER, CCRF-CEM, and 293. In HELA, only one band was detected (at M_r 35,000), whereas in HPB-ALL, no signals were observed. The detection of *TLX1* by Western blot is depicted in *D*. In contrast to HPB-ALL, CCRF-CEM, and PEER, ALL-SIL shows a protein band at M_r ~35,000 (*arrow*). Thus, transcription of *TLX1* in PEER proceeded without translation. Alternative splicing of *BCL11B* (shown in *E*) was analyzed by RT-PCR using specific primers for variant 1 (*middle*) or variant 2 (*bottom*). Excepting PEER, all cell lines shown expressed *BCL11B* variant 2 (*arrow*). Variant 1 was expressed in cell lines with t(5;14)(q35.1;q32.2): BE-13, CCRF-CEM, HPB-ALL, and MKB1 (*arrow*). Both variants were expressed in the peripheral blood of a healthy donor. cDNA quality was checked using primers for *UBTF* (*top gel*).

and BE-13 (Table 2). Even HPB-ALL, which carries the "standard" t(5;14) (5), lacked *NKX2–5* expression. Two nonhematopoietic cell lines (HELA and 293) also expressed *NKX2–5* (Table 2), but FISH analysis confirmed absence of any t(5;14) therein (*data not shown*). The extent to which deregulated homebox genes carry mutations, as reported in *PAX5* after somatic hypermutation (14), remains unclear; cloning and sequencing of the full-length *NKX2–5* cDNA from PEER derived by PCR indicated the absence of point mutations. Northern analysis of *NKX2–5* in CCRF-CEM and PEER revealed at least two mRNA species migrating at 1.8 and 3.6 kb, respectively (Fig. 2*B*). This finding is consistent with the data of Shiojima *et al.* (15) and might represent alternate splicing, polyadenylation, or some other cause. To investigate protein expression, we analyzed *NKX2–5* by Western blotting. CCRF-CEM, PEER, and 293 all yielded *NKX2–5* protein at M_r ~35/37,000, corresponding to phosphorylated and unphosphorylated isoforms (16), whereas HELA yielded a single species

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Table 2 *Gene expression in T-ALL and other cell lines*

Data show expression as measured by RT-PCR in T-ALL cell lines and in those representing other hemic and solid tumors. +, −, ( ) indicate expression detected, not detected, not tested.

	TLX1	*TLX3*	*NKX2–5*	*BCL11B(2)*	*BCL11B(1)*
T-ALL					
ALL-SIL	+	−	−	−	
BE-13	−	−	+	+	+
CCRF-CEM	−	−	+	+	+
DU-528	−	−	−	−	
HPB-ALL	−	+	−	+	+
HSB-2	−	−	−	−	
JURKAT	−	−	−	+	−
KARPAS-45	−	−	−	+	−
KE-37	−	−	−	−	
LOUCY	−	−	−	−	
MHH-TALL-1	−	−	−	−	
MHH-TALL-2	−	−	−	−	
MKB-1	−	−	+	+	+
MOLT-4	−	−	−	−	
MOLT-13	−	−	−	+	−
MOLT-16	−	−	−	−	−
P12-ICHIKAWA	−	−	−	−	
PEER	+	−	+	−	−
PF-382	−	−	−	+	−
RPMI-8402	−	−	−	−	
SUP-T1	−	−	−	−	
TALL-104	−	−	−	−	
Other (hemic)					
HC-1	−	−	−		
HDLM-2	−	−	−		
HL-60	−	−	−	−	
K-562	−	−	−		
L-428	−	−	−		
MHH-PreB-1	−	−	−		
MEG-01	−	−	−	+	−
NC-NC	−	−	−	−	
NK-92	−	−	−	−	
SUP-M2	−	−	−		
TF-1	−	−	−		
U-698-M	−	−	−	−	
YT	−	−	−	−	
Other (solid)					
293	−	+	+	−	−
42-MG-BA	−	−	−	−	
EFM-192B	−	−	−		
H-1339	−	−	−		
HELA	−	+	+	−	
HT-1080	−	−	−	−	
KYSE-70	−	−	−		
MHH-ES-1	−	−	−		
PC-3	−	−	−		
RH-30	−	−	−	−	
SIMA	−	−	−	−	
Y-79	−	−	−	−	

only which migrated at 35 kDa (Fig. 2*C*). The strongest protein signals were present in CCRF-CEM, consistent with the Northern analysis data.

Analysis of Genes Located in the Breakpoint Region at 14q32. Unlike the 5q35.1 breakpoints in CCRF-CEM and PEER, those at 14q32.2 lay out with any obvious nearby target. Instead, like those juxtaposing *TLX3* (1, 5), they were scattered over 1 Mb distal (centromeric) of *BCL11B*, a zinc-finger transcription factor preferentially expressed in T cells (4, 5). The immediately centromeric gene, *HERV-HD1/HSU88895*, an endogenous retrovirus known to mediate ectopic gene expression in T cells (17), lies just telomeric of the breakpoint cluster. Centromeric of the breakpoint cluster, the nearest annotated gene is *VRK1*, which regulates p53 by phosphorylation (18). RT-PCR analysis showed that V*RK1* and *HSU88895* were expressed in both CCRF-CEM and PEER (*data not shown*) and in most hematopoietic and solid tumor cell lines examined (5), whereas *BCL11B* was preferentially expressed in T-ALL cell lines (8 of 22) but rarely in other hematopoietic (1 of 6) or solid tumor (0 of 7) cell lines (Table 2). Interestingly, *BCL11B* was expressed in HPB-ALL and CCRF-CEM but not in PEER. In addition, two alternative splicing products (variants 1 and 2, NM_138576/022898, respectively) were analyzed by RT-PCR (Fig. 2*E*; Table 2). Variant 1 was only detectable in cell lines with t(5;14). Nine breakpoints at 3′-*BCL11B* have now been mapped, broadcast over circa 1 Mb (Fig. 1*F*). The cytogenetic data imply that the putative regulatory region lies centromeric of this cluster, bounded by PEER at 92.2 Mb.

Screening of T-ALL Cell Lines for NK-like Homeobox Gene Expression. *NKX2–5*, as well as *TLX1* and *TLX3* whose expression promotes T-ALL, belongs to the NK-like family of homeobox genes (6), first identified in *Drosophila* (19). NK-family members exhibit conservation of specific sequences within the homeodomain. In addition, most NK-homeobox genes, including *NKX2–5*, *TLX3*, and *TLX1*, contain so-called TN- and NK2-domains in their respective NH_2- and COOH-terminal regions (20). Hitherto, 14 NK-like homologues have been described in *Homo sapiens* (Table 1). To study the expression of other members of this group in T-ALL, a clue to ectopic activation, we screened 22 T-ALL cell lines by RT-PCR (Table 2). In addition to *NKX2–5*, PEER, but not BE-13, expressed *TLX1* mRNA (Fig. 2*A*) without any cytogenetic rearrangement of *TLX1*, whether analyzed conventionally or by FISH using flanking clones (data not shown). However, *TLX1* protein was undetectable in PEER (Fig. 2*D*). No additional T-ALL cell line expressed any of the 11 other NK-like homologue tested. Thus, *TLX1* (ALL-SIL, K3P, PER-255, SUP-T4), *TLX3* (HPB-ALL), and *NKX2–5* (PEER, CCRF-CEM) were the only NK-like homeobox genes both expressed and translated in T-ALL cell lines (8).

DISCUSSION

We have identified a novel variant t(5;14)(q35.1;q32.2) in pediatric T-ALL cells, whereby *NKX2–5* is activated instead of *TLX3*, thereby adding *NKX2–5* to the list of leukemogenic homeobox genes. Although mutations of *NKX2–5*, the human structural and functional homologue of the Drosophila *tinman* which specifies cardiac muscle progenitors in nascent mesoderm, cause atrial septal heart defects, expression has yet to be recorded in neoplastic tissue. *NKX2–5* shares with *TLX1* embryonal expression in spleen (12, 21, 22), and both promote cell survival (22, 23). This may point to a similar genetical program controlled by NK-like homeobox genes, reactivation of which may promote development of T-cell leukemia, contrasting with NK genes involved in solid tumors, which are usually down-regulated.

Contrasting models may be invoked to explain activation of *NKX2–5* or *TLX3* in T-ALL by t(5;14). First, chromosome rearrangement may remove upstream negative regulators, as reported for *TLX1* (24). Or, their removal may target silencing of tissue-preferential regulators (25). Alternately, (a) long-range tissue-specific distal-*BCL11B* regulatory element(s) enhance *NKX2–5* promoter activity by chromosomal juxtaposition. The last model explains the consistent recurrence of t(5;14) in T-ALL and also permits analogy of 3′-*BCL11B* with *IGH*-enhancers, as implied by the data of Hansen-Hagge *et al.* (7). Although the homologous *BCL11A* is a dominant oncogene (4), the leukemogenic role of *BCL11B*, as evidenced by its nonexpression in PEER, remains unclear. Although neighboring genes, including *HSU88895* and *VRK1* as well as interspersed putative genes (UniGene Clusters), either strain the breakpoint data or fail to exhibit preferential expression in T cells (5), it would be premature to abandon their candidacy in favor of *BCL11B*.

This report identifies pediatric T-ALL cell lines with a variant t(5;14) in which a novel homeodomain target, *NKX2–5*, is ectopically activated. Interestingly, the affected cell lines derive from female patients contrasting with the strong male preference hitherto observed

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in standard t(5;14) T-ALL (1, 3). Pediatric T-ALL samples will require testing for both *NKX2–5* and *TLX3* expression and/or modified FISH analysis to ascertain its clinical incidence, gender preference, and lineage associations. Preliminary data suggest that variant t(5;14) occurs more rarely than the standard translocation. Assessment of *NKX2–5* as a potential new therapeutic target is currently underway in this lab. In addition to modeling a specific subtype of pediatric T-ALL, the cell lines described in this report should aid investigation of ectopic, NK-like homeodomain gene expression, and the nature of their putative activator(s) at 14q32.2, which appears to represent an emerging new pathological mechanism in T-cell leukemia.

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