Leukemia (2008) 22, 600–607

www.nature.com/leu

ORIGINAL ARTICLE

MEF2C is activated by multiple mechanisms in a subset of T-acute lymphoblastic leukemia cell lines

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In T-cell acute lymphoblastic leukemia (T-ALL) the cardiac homeobox gene NKX2-5 (at 5q35) is variously deregulated by regulatory elements coordinating with BCL11B (at 14q32.2), or the T-cell receptor gene TRD (at 14q11.2), respectively. NKX2-5 is normally expressed in developing spleen and heart, regulating fundamental processes, including differentiation and survival. In this study we investigated whether NKX2-5 expression in T-ALL cell lines reactivates these embryonal pathways contributing to leukemogenesis. Among 18 known targets analyzed, we identified three genes regulated by NKX2-5 in T-ALL cells, including myocyte enhancer factor 2C (MEF2C). Knockdown and overexpression assays confirmed MEF2C activation by NKX2-5 at both the RNA and protein levels. Direct interactions between NKX2-5 and GATA3 as indicated by co-immunoprecipitation data may contribute to MEF2C regulation. In T-ALL cell lines LOUCY and RPMI-8402 MEF2C expression was correlated with a 5q14 deletion, encompassing noncoding proximal gene regions. Fusion constructs with green fluorescent protein permitted subcellular detection of MEF2C protein in nuclear speckles interpretable as repression complexes. MEF2C consistently inhibits expression of NR4A1/NUR77, which regulates apoptosis via BCL2 transformation. Taken together, our data identify distinct mechanisms underlying ectopic MEF2C expression in T-ALL, either as a downstream target of NKX2-5, or via chromosomal aberrations deleting proximal gene regions.
Leukemia (2008) **22,** 600–607; doi:10.1038/sj.leu.2405067;
published online 13 December 2007
Keywords: t(5;14); NK-like; microdeletion

Introduction

Homeobox genes code for transcription factors impacting basic cellular processes, such as differentiation, proliferation and apoptosis.[1] If mutated or dysregulated many examples have been shown to promote oncogenesis in both solid and hematopoietic tumors.[2,3] With respect to evolutionary history, homeobox genes have been classified as clustered or nonclustered according to their genomic localization, and in subfamilies according to their homeobox sequences.[4,5] In T-cell acute lymphoblastic leukemia (T-ALL) genes belonging to both clustered (HOXA) and the subfamily of NK-like homeobox genes exhibit pathological involvement.[6–8] The NK-like subfamily includes TLX1, TLX3 and NKX2-5, all ectopically activated in T-ALL cells via chromosomal rearrangements.[7,9,10]

In T-ALL expression of certain oncogenes including homeobox genes TLX1 and TLX3 define disease subtypes.[11,12] Data obtained by expression profiling link these T-ALL subtypes to developmental staging of T cells, indicating specific differentiation arrest.[13] T-ALL cases expressing TLX3 have poorer prognoses than those expressing TLX1 instead,[13,14] suggesting differences in downstream regulation between these two closely related homeobox genes. With few exceptions, ectopic expression of NK-like homeobox genes is activated via chromosomal aberrations.[15] For all three homeobox genes TLX1, TLX3 and NKX2-5 juxtaposition to TRD at 14q11 has been described. Additionally, TLX1 is activated in t(7;10)(q35;q24) via fusion with TRB, and both, TLX3 and NKX2-5 by t(5;14)(q35;32) involving BCL11B.[7,9,10,16–19]

TLX3 and NKX2-5 are located at 5q35 separated by approximately 2 Mb. In t(5;14)-positive cells orphan enhancer elements originated from far downstream regions of BCL11B at 14q32 are juxtaposed with either homeobox gene.[7] These elements co-localize to DNAse I hypersensitive sites, contain acetylated histones, bind to the nuclear matrix and are enriched in HMGA1-binding sites juxtaposed by t(5;14) rearrangements with PU.1 sites located in the promoter regions of both TLX3 and NKX2-5.[20] Treatment with oligonucleotides matching enhancer elements at 3′-BCL11B effected downregulation of NKX2-5 in T-ALL PEER cells where this gene is juxtaposed to BCL11B by t(5,14).[20]

In the present study this enhancer inhibition assay was used to effect NKX2-5 knockdown in T-ALL cells therein to evaluate candidate downstream targets of this gene previously identified in other tissues, notably developing spleen and heart.[21] We report aberrant myocyte enhancer factor 2C (MEF2C) expression, which may implicate analogous activation mechanisms in T-ALL cell lines. In addition we show that a 5q14 deletion targets loss of putative regulatory regions associated with aberrant expression of the MEF2C transcription factor recently shown to act as a cooperating oncogene in mouse leukemia together with SOX4 in an insertional retrovirus assay.[22] Our data highlight the MEF2C transcription factor as a new dysregulatory target in T-ALL subsets and provide a new example of a putative oncogenic microdeletion.

Materials and methods

Cell lines and treatments

Detailed descriptions of cell lines used in this study and their culture methods are given in Drexler (2005) and are accessible at www.dsmz.de.[23] Electroporation of PEER was performed as previously described.[20] Transfection of HeLa with plasmid DNA and siRNA oligonucleotides was performed according to the manufacturers' instructions (Qiagen, Hilden, Germany). Trichostatin-A (TSA) and p38 inhibitor SB203580 were obtained from Sigma (München, Germany), and the BCL2 inhibitor YC137 from Calbiochem (Darmstadt, Germany).

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Received 4 June 2007; revised 9 November 2007; accepted 16 November 2007; published online 13 December 2007

Analysis of gene expression
RNA preparation and reverse transcription (RT)–PCR analysis were performed as previously described.[7,20] Oligonucleotides used for RT–PCR are listed in Supplementary Table 1. Quantitative RT-PCR analysis was performed with a 7500 Real-Time PCR System (Applied Biosystems, Darmstadt, Germany) using Taqman gene expression assays for MEF2C or NR4A1 in comparison to TBP obtained from Applied Biosystems.

Chromosome analysis
Methods used for karyotyping and fluorescence *in situ* hybridization (FISH) are detailed elsewhere.[24,25] Fosmid clones (G248P8626G4 named here D1 and G248P87254D4 named here D2) and RP11 library bacterial artificial chromosome (BAC) clones (484D1 and 293L18) were obtained from the Sanger Centre (Cambridge, England). Copy numbers of proximal (exon 1d) and distal (exon 2) gene regions of MEF2C were determined by real-time PCR (see above) using an SYBR Green-based detection system (Applied Biosystems).

Western blot and immunoprecipitation
Western blot analysis was performed as previously described.[7] Antibodies used for detection of MEF2C, ERK1/2, NKX2-5 and GATA3 were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The same antibodies were used for immunoprecipitation, and chromatin immunoprecipitation (ChIP) performed as described elsewhere.[26,20]

Fluorescence microscopy
Enhanced green fluorescent protein (EGFP) constructs were introduced in PEER and HeLa cells by electroporation and transfection, respectively, as described above. Detection of fluorescence in cytospins was performed as previously described.[27]

Analysis of apoptosis
Measurement of apoptosis was performed by flow cytometry as previously described.[28]

Results and discussion

Screening of NKX2-5 target genes
To investigate whether NKX2-5 reactivates embryonal cardiac/splenic signaling in T-ALL cells, we performed expression analysis in t(5;14)(q35;q32) cell lines PEER and CCRF-CEM[7] for 18 known targets of NKX2-5/tinman: CALR, CITED2, CRIPTO, FOXH1, GATA4, GATA5, GATA6, HAND2, HOP, JMJD2A, MEF2C, MEIS2, MOV10L1, NR2F1, NR2F2, NR2F6, PITX2 and TBX5.[29–39] Most genes were neither expressed in PEER nor in CCRF-CEM as analyzed by RT–PCR. However, five genes (CALR, CRIPTO, JMJD2A, MEF2C and PITX2) were expressed in both cell lines, while NR2F2 was expressed in PEER only, indicating their potential activation by NKX2-5. To examine the role of NKX2-5 in the expression of the 5 co-expressed genes, NKX2-5 knockdown experiments were performed in PEER by enhancer inhibition.[20] Here we used double stranded oligonucleotide DSO6 previously shown to inhibit NKX2-5 exclusively, while expression levels of neighboring genes, BCL11B and BC043585, remain unperturbed.[20] Reduction of NKX2-5 protein expression was confirmed by western blotting (Figure 1a). Expression analysis showed downregulation of PITX2 and MEF2C and upregulation of CRIPTO after oligonucleotide treatment, while CALR and JMJD2A remained unmodulated (Figure 1b). These data suggest that NKX2-5 activates PITX2 and MEF2C, inhibits CRIPTO, while CALR and JMJD2A are not subject to regulation. Recent data on the NKX2-5 orthologue tinman indicate it functions as a transcriptional activator or repressor, depending on the occurrence of interactions with co-activator or corepressor, respectively.[40] However, CRIPTO is reportedly overexpressed in certain tumor types,[41] rendering a less plausible target for downregulation by NKX2-5. Both PITX2 and MEF2C are involved in hematopoiesis,[42,43] qualifying both of these as potential leukemogenic factors.

NKX2-5 activates MEF2C
Here we focused analysis on MEF2C which has been recently identified using insertional mutagenesis assays as a cooperating oncogene together with SOX4 in mouse leukemogenesis.[22] MEF2C is a member of the MADS box family of transcription factors. Expression of both MEF2C and MEF2D occurs in lymphocytes, while that of MEF2C is restricted to B-cells, implying that activation in T-ALL cells may require dysregulation.[43] In pre-B-ALL, t(1;19)(q23;p13) results in the fusion of MEF2D with DAZAP1 shown to be involved in pathogenesis,[44,45] supporting the notion of leukemic potential among MEF2-proteins.

Expression of MEF2C and MEF2D was analyzed in additional T-ALL cell lines by RT–PCR (Table 1). All 23 T-ALL cell lines tested positive for MEF2D and 10/23 (43%) for MEF2C, demonstrating the physiological expression of MEF2D in T-cells and suggesting ectopic expression of MEF2C in both NKX2-5-positive and -negative T-ALL cells. The absence of MEF2C expression in cell lines expressing TLX1 (ALL-SIL) or TLX3 (DND-41, HPB-ALL) pinpoints differences between T-ALL subtypes expressing NK-like homeobox genes and supports the specificity of activation by NKX2-5.

For overexpression analysis NKX2-5 cDNA was cloned into expression vector (pIRES2-EGFP) to generate the construct pNKX2-5. PEER cells transfected with pNKX2-5 by electroporation showed consistently enhanced MEF2C expression, (Figure 1c). PEER cells sustaining NKX2-5 knockdown by enhancer inhibition and RNAi were analyzed quantitatively for MEF2C expression by real-time PCR (Figure 1d). Resultant data corresponded to qualitative PCR results, confirming the activatory input of NKX2-5.

del(5)(q14) activates MEF2C
To analyze if chromosomal aberrations contribute to MEF2C expression, we cytogenetically screened T-ALL cell lines for chromosomal breaks near the MEF2C locus at 5q14. LOUCY carries del(5)(q14q35) as detected by G-banding (Supplementary Figure 1a) and chromosome painting (Supplementary Figure 1b) and expresses MEF2C (Table 1). By FISH analysis we mapped the breakpoint at 5q14 between G248P fosmids 8626G4 and 87254D4, corresponding to intron 3 of the MEF2C gene (Supplementary Figure 1c and d). This aberration deletes alternate noncoding exons 1b-d juxtaposing the coding part of the gene to hitherto unknown sequences. Painting analysis contraindicated insertion of material from other chromosomes, suggesting that MEF2C fuses with telomeric 5q regions (Supplementary Figure 1b). Supporting SNP array data published for LOUCY (www.Sanger.com) indicated incomplete loss of 5q14-35 via fractionated deletions leaving residual material at

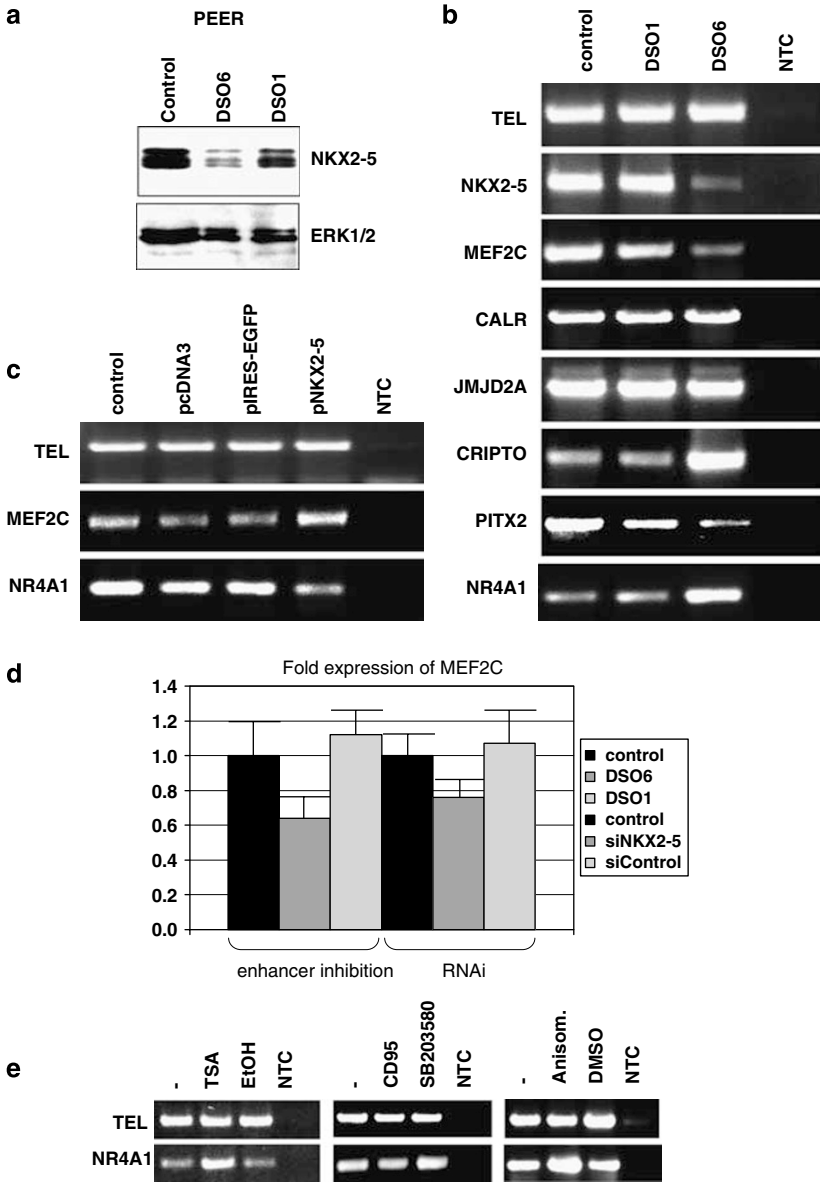

Figure 1 Gene expression analysis. (**a**) Western blot analysis of PEER cells treated with DSO6 enhancer-inhibitory oligonucleotides confirms downregulation of NKX2-5 protein expression. Antibody-detecting MAPK ERK1/2 was used as loading control. (**b**) PEER cells subjected to enhancer inhibition were subsequently analyzed for target gene expression: oligonucleotide DSO6 reduces NKX2-5 expression, DSO1 serves as control.[20] Note downregulation of MEF2C and PITX2 and upregulation of CRIPTO and NR4A1 by NKX2-5 knockdown. (**c**) Overexpression of NKX2-5 in PEER cells forced by construct pNKX2-5 effects upregulation of MEF2C and downregulation of NR4A1. Untreated cells or cells electroporated with empty vectors served as controls. (**d**) Real-time PCR analysis of MEF2C expression of PEER cells electroporated with DSOs for enhancer inhibition or synthetic siRNAs for RNAi-mediated NKX2-5 knockdown confirms activatory input of NKX2-5. (**e**) Pharmacological treatment of PEER cells for 16 h with 500 μM HDAC inhibitor TSA, 10 μg ml^{-1} activatory antibody directed against CD95, 100 μM MAPK p38 inhibitor SB203580 and 10 μM p38 activator anisomycin. Note NR4A1 upregulation after treatments with either TSA or anisomycin, while CD95 and SB203580 treatments proved ineffectual. Expression of TEL served as positive control. NTC, no template control.

both 5q15 and 5q31-32. On der(5) both the deletion of 5q14 (corresponding to 88.2–93.0 Mb) and the presence of 5q15 material (94–95 Mbp) was confirmed by FISH using RP11 BAC clones 6N12, 68F17, 120I14, 241L8, 484C12, 33A7 and 126G10 (data not shown). The non-deleted region contains the gene C5ORF36 which was strongly expressed in 8/8 T-ALL cell lines including LOUCY, as analyzed by RT–PCR (data not shown), potentially mediating an activatory influence on MEF2C expression via juxtaposition. In other T-ALL cell lines expressing MEF2C (JURKAT, KE-37, MOLT-14, P12-ICHIKAWA, RPMI-8402, SUP-T1), no chromosomal aberrations nearby MEF2C were detected as analyzed by FISH using RP11 BAC clones 484D1 (located at 87.5 Mb) and 293L18 (located at 88.5 Mb) as probes (data not shown).

Screening for microdeletions was performed by quantitative real-time PCR amplifying exon 1d and exon 2 (Supplementary Figure 1d) from genomic DNA of all MEF2C expressing cell lines. Resulting data confirm loss of proximal MEF2C exons in LOUCY and probably demonstrate a cytogenetically silent deletion of exon 1d in RPMI-8402 (Supplementary Figure 1e). SNP array data (www.Sanger.com) for RPMI-8402 give no hint

Table 1 Gene expression analysis by RT–PCR

	NKX2-5	*MEF2C*				*MEF2D*	*BCL2*	*BCLB*	*BFL1*
		(2–3)	*(1a–3)*	*(1b–1a)*	*(1d–1a)*				
ALL-SIL	–	–				+			
CCRF-CEM	+	+	+	+	–	+	+	–	–
CML-T1	–	–				+			
DND-41	–	–				+			
HD-MAR	–	–				+			
HPB-ALL	–	–				+			
H-SB2	–	–				+			
HT-1	–	–				+			
JURKAT	–	+	+	–	–	+	+	–	–
KARPAS-45	–	–				+			
KE-37	–	+	+	+	–	+	+	–	–
LOUCY	–	+	+	–	–	+	+	+	+
MHH-TALL2	–	–				+			
MOLT-4	–	–				+			
MOLT-14	–	+	+	+	–	+	+	–	–
MOLT-16	–	+	+	+	–	+	+	+	+
P12-ICHIKAWA	–	+	+	–		+			
PEER	+	+	+	–	–	+	+	–	–
PF-382	–	–				+			
RPMI-8402	–	+	+	+	–	+	–	–	–
SUP-T1	–	+	+	+	–	+	+	–	–
TALL1	–	–				+			
TALL-104	–	–				+			
HeLa	+	+					+	+	

Gene expression data of 23 T-ALL cell lines in addition to HeLa as analyzed by RT–PCR. MEF2C expression was analyzed for different exon combinations. Positive expression is indicated by (+), negative by (–).

of 5q14 deletion. However, these results imply frequent deletion of the affected region in T-ALL cells resulting in ectopic expression of MEF2C. Due to size differences of the deleted fragments we speculate that loss of repressor elements instead of juxtaposition of enhancers contribute to MEF2C activation, implying that regulatory microdeletions may play a wider oncogenic role than hitherto suspected.

RT–PCR analysis of alternate first exons expressed in T-ALL cell lines detected transcription of exon 1a (containing the start codon) in all MEF2C-positive cell lines. Seven of them additionally express exon 1b but none expresses exon 1d (Table 1). For LOUCY this is in accordance with genomic deletion of exons 1b-d on der(5). In contrast to upstream regions of exon 1d, those of exons 1a and 1b contain potential binding sites for NKX2-5 (UCSC genome bioinformatics), supporting direct activation of MEF2C by NKX2-5 in PEER and CCRF-CEM (Table 1). However, for the remaining MEF2C-positive cell lines other activation mechanism(s) must be invoked, perhaps involving basic helix-loop-helix proteins, ETS factors or LIM-homeodomain factors.[46–48]

Protein analysis of MEF2C, GATA3 and NKX2-5

Western blot analysis detected MEF2C protein expression in both PEER and LOUCY in contrast to DND-41 (Figure 2a), confirming RT–PCR results (Table 1). PEER cells electroporated with inhibitory oligonucleotides reducing NKX2-5 expression showed decreased levels of MEF2C protein (Figure 2b), according to the results obtained by real-time PCR analysis (Figure 1d). In *Drosophila* mef2 is regulated by the NKX2-5 ortholog tinman together with the GATA ortholog pannier.[35,36] In vertebrates several GATA homologous are known,[49] among which GATA4 interacts with NKX2-5 and GATA3 is expressed specifically in T-cells.[50,51] Accordingly, in five T-ALL cell lines analyzed we were able to detect GATA3 protein by western blotting (Figure 2c). Subsequently, we examined protein interactions between NKX2-5 and GATA3 in T-ALL cells (PEER and CCRF-CEM) by immunoprecipitation (Figure 2d) using antibodies previously tested in western blots.[7] These data indicate direct interactions which may contribute to MEF2C activation. To confirm binding of NKX2-5 to the MEF2C gene we performed ChIP analysis using NKX2-5 antibody. PCR analysis of subsequently isolated DNA-demonstrated amplification of MEF2C sequences located upstream of exon 1a containing a potential NKX2-5-binding site in contrast to MEF2C exon 9 sequences used as control (Figure 2e). These data indicate direct binding of NKX2-5 at its consensus site upstream of exon 1a.

MEF2C represses expression of NR4A1

We then analyzed the subcellular distribution of MEF2C protein in T-ALL cells. Therefore, MEF2C cDNA was cloned in fusion with EGFP, generating an expression construct (pMEF2C-EGFP), which was electroporated into PEER cells. Images obtained by fluorescence microscopy indicate a distribution concentrated as nuclear speckles in contrast to a pEGFP control, which showed even cytoplasmic distribution (Figure 3). The same distribution pattern was observed after transfection of pMEF2C-EGFP into 293 or HeLa cells, indicating absence of tissue or cell specificity (data not shown). MEF2C-EGFP fusion construct lacking the terminal exon 9 of MEF2C (pMEF2Cd9-EGFP) showed cytoplasmic distribution comparable to that of the pEGFP control (Figure 3). This is in accordance with a nuclear localization sequence identified in this region.[52] Recently, MEF2 proteins have been shown to interact with histone deacetylases (HDACs) via NCOR2, mediating gene repression. In addition, co-localization experiments analyzing MEF2C together with NCOR2 indicated presence in nuclear speckles and supported importance of this distribution for gene repression activity.[53]

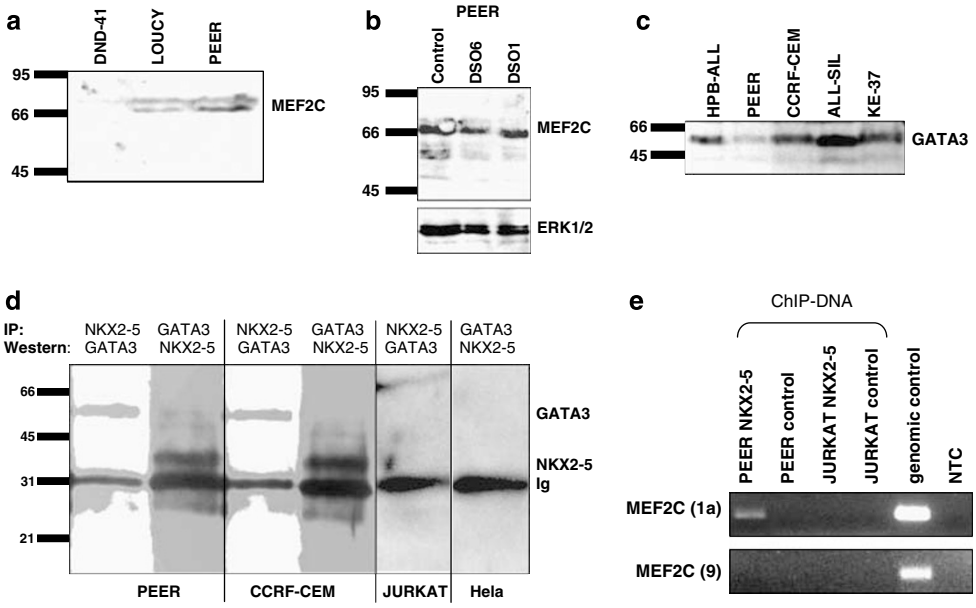

Figure 2 Protein analysis. (**a**) Using MEF2C-specific antibody characteristic ~66 kDa signals were detected in T-ALL cell lines, LOUCY (5q-) and PEER (NKX2-5 activation), but not in DND-41 (TLX3 activation). (**b**) PEER cells treated by enhancer inhibition effecting NKX2-5 knockdown were analyzed by western blotting for MEF2C expression. Treatment with DSO6 resulted in decreased levels of MEF2C protein, demonstrating that NKX2-5 stimulates MEF2C expression. As loading control antibody detecting MAPK ERK1/2 was used. (**c**) Western blot analysis of GATA3 in T-ALL cell lines detects a protein of ~50 kDa. (**d**) Immunoprecipitation followed by western blot analysis using antibodies detecting NKX2-5 and GATA3, respectively, in PEER and CCRF-CEM cells. Control cell lines JURKAT (expression of GATA3 but not of NKX2-5) and HeLa (expression of NKX2-5 but not of GATA3) served as negative controls and confirm specificity of the used antibodies. Data indicate physical interaction between NKX2-5 and GATA3 proteins in T-ALL cells. (**e**) PCR analysis of MEF2C sequences located upstream of exon 1a and within exon 9, respectively. DNA was obtained by ChIP of PEER and JURKAT cells using NKX2-5 or control antibodies. Data indicate direct interaction of NKX2-5 with MEF2C sequences upstream of exon 1a.

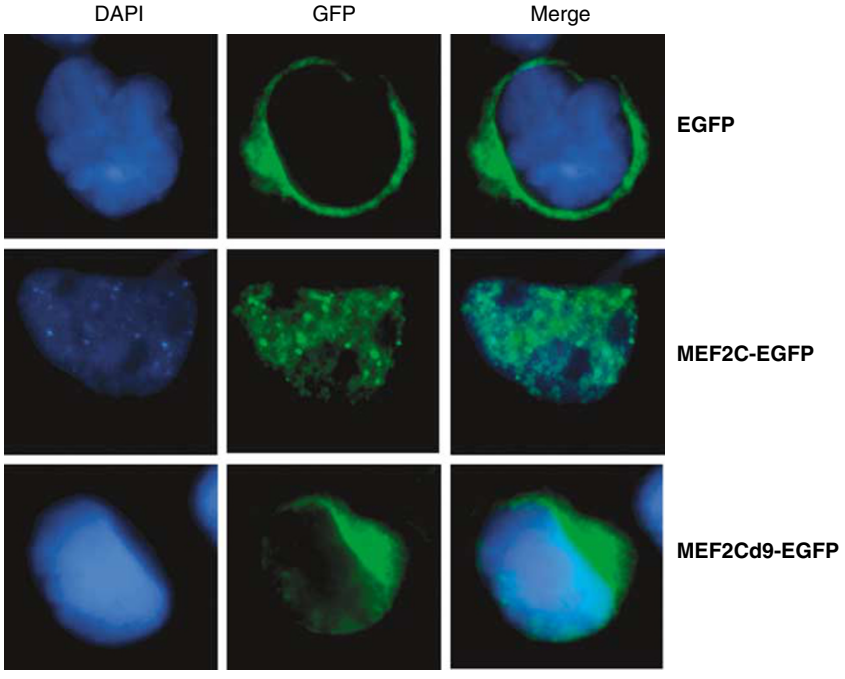

Figure 3 Fluorescence microscopy of enhanced green fluorescent protein (EGFP) fusion proteins. PEER cells were electroporated with expression vectors containing EGFP, MEF2C-EGFP or MEF2Cd9-EGFP, lacking distal exon 9. Images show 4-6-diamidino-2-phenylindole (DAPI)-stained nuclei (blue), green-labeled EGFP and the merged data. Note the speckled distribution pattern of MEF2C-EGFP in the nucleus.

Therefore, the speckled pattern of MEF2C observed here in T-ALL cells suggests involvement in a repression complex comprising NCOR2 and HDAC.

We then investigated the consequences of MEF2C expression for downstream genes in T-ALL cells. Genes positively regulated by MEF2C, such as DLX5 and DLX6,[54] were not detectable in

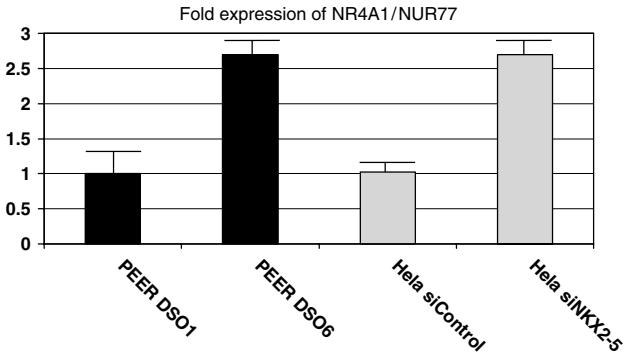

Figure 4 Real-time PCR analysis of NR4A1 expression. For NKX2-5 knockdown PEER cells were treated by enhancer inhibition and HeLa cells by RNAi using siRNA directed against NKX2-5. In both PEER and HeLa, reduced expression of NKX2-5 results in increased expression of NR4A1. Standard deviations are indicated by bars.

Table 2 Analysis of apoptosis

Cell line	*Treatments*		*Apoptotic cells (%)*
PEER	DSO6	—	21
	DSO1	—	21
HeLa	siControl	—	4
	siNKX2-5	—	4
	siControl	YC137	12
	siNKX2-5	YC137	19

PEER and HeLa cells were treated as indicated, stained by propidium iodide and analyzed by flow cytometry, determining the fraction of apoptotic cells.

PEER as analyzed by RT–PCR (data not shown), suggesting inability to activate these physiological targets. During thymic T-cell development massive apoptosis of thymocytes occurs, a process which involves the activity of the NUR77 subfamily of orphan nuclear receptors.[55,56] MEF2D has been shown to activate apoptosis during T-cell development via NR4A1/NUR77[57–59]—a process probably dysregulated by MEF2C. Therefore, overexpression and knockdown of NKX2-5 was performed in PEER and HeLa cells (which also express NKX2-5),[7] which were subsequently analyzed for NR4A1 expression. PEER cells were electroporated as described above, HeLa cells were transfected with pNKX2-5 and siRNA oligonucleotides directed against NKX2-5, respectively. Real-time PCR analysis confirmed RT–PCR data on NR4A1 expression as shown in Figures 1b and c and 4. In both cell lines, NR4A1 expression was downregulated by overexpression, but upregulated by knockdown of NKX2-5, indicating NR4A1 silencing via NKX2-5/MEF2C. A recent study has identified another family member, NR4A3, as activatory target of TLX1, which may contribute to the good prognosis of that T-ALL subtype.[55,60,61]

Further evidence that NKX2-5/MEF2C represses NR4A1 was obtained by pharmacological treatments followed by expression analysis. First, treatment with the histone deacetylase inhibitor TSA, previously shown to reduce NKX2-5 expression in PEER cells, effected NR4A1 upregulation (Figure 1e).[20] However, a reported association of MEF2C with HDACs may have played a contributory role.[52,62] Second, phosphorylation of MEF2C (but not of MEF2D) by MAPK p38 transforms the factor into a transcriptional activator.[63] Accordingly, treatment of T-ALL cell lines with the p38 activator anisomycin effected NR4A1 upregulation, unlike treatment with the p38 inhibitor SB203580 (Figure 1e), indicating absence of p38 activity in untreated cells. Taken together, our data suggest that ectopically expressed MEF2C is located in a repressor complex and mediates inhibition of NR4A1.

NKX2-5 mediates inhibition of apoptosis

It has been shown that the apoptotic activity of NR4A1 functions by transforming BCL2 proteins from anti- into pro-apoptotic factors.[64] NR4A1 binds BCL2 family members BCL2, BCLB and BFL1.[65] RT–PCR analysis showed ubiquitous expression of BCL2 contrasting with BCLB and BFL1 (Table 1), pinpointing BCL2 as both, a prominent survival factor and a potential target of NR4A1 in T-ALL cells. For functional analysis concerning NR4A1 expression both, PEER and HeLa cells were treated for 20 h with 5 μg ml^{-1} BCL2 inhibitor Y137 resulting in reduced viability of both cell lines (data not shown).[66] PEER and HeLa treated for NKX2-5 knockdown by enhancer inhibition or siRNA, respectively, were subsequently analyzed after 20 h by flow cytometry for apoptosis but showed no significant difference in comparison to control cells. However, transfected HeLa cells additionally stimulated with YC137 showed significant increase of apoptosis accompanied by reduced NKX2-5 expression (Table 2). Since BCL2 has been shown to mediate survival of thymocytes,[67,68] ectopic expression of NKX2-5/MEF2C may promote leukemogenesis by inhibiting apoptosis. Accordingly, in acute myeloid leukemia both MEF2C[69] and NR4A1[70] have been identified as an oncogene and tumor suppressor gene, respectively, supporting their leukemic potential.

Taken together, we have identified multiple mechanisms underlying ectopic MEF2C activation in T-ALL cells, either via NKX2-5 signaling, or by chromosomal deletion at 5q14. MEF2C activation by NKX2-5 underlines both the impact of this homeodomain protein on leukemogenesis and the stratification among members of the NK-like family of homeobox genes distinguishing this member from TLX1/3. MEF2C probably inhibits BCL2-regulated apoptosis by repression of NR4A1, promoting survival of leukemic cells. These results both demonstrate functional characteristics of T-ALL cells expressing MEF2C with potential therapeutic implications for treatment with HDAC inhibitors and support more detailed genomic scrutiny of regulatory regions in oncogenesis.

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Supplementary Information accompanies the paper on the Leukemia website (http://www.nature.com/leu)