TLX1 induced T-cell acute lymphoblastic leukemia

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ABSTRACT

The *TLX1* transcription factor oncogene is frequently activated by chromosomal translocations in T-cell acute lymphoblastic leukemia (T-ALL) and defines a distinct molecular group of tumors characterized by differentiation arrest at the early cortical stage of thymocyte differentiation and excellent response to therapy. Recent developments from the analysis of genomic data on TLX1 specific transcriptional targets and analysis of the molecular mechanisms of TLX1 transformation in human and mouse induced leukemias have shown novel insight on the activity of this transcription factor oncogene. Aberrant expression of TLX1 in T-cell progenitors disrupts normal T-cell development and triggers the development of aneuploidy during T-cell transformation. Importantly, the disruption of the mitotic checkpoint in TLX1 induced tumors may be linked not only to the acquisition of secondary genetic alterations in T-ALL but also may be increased sensitivity of these tumors to chemotherapy with drugs targeting the formation of the mitotic spindle.

BACKGROUND

T-ALL is an aggressive hematologic cancer caused by malignant transformation of developing T-cells. The *TLX1* transcription factor oncogene is the target of translocation t(10;14)(q24;q11) in 5% to 10% of pediatric and up to 30% of adult T-ALL cases (1-4). This translocation juxtaposes an intact *TLX1* gene to the very strong enhancer elements of the T-cell receptor loci, resulting in aberrantly high *TLX1* expression levels.

TLX1 (also known as *HOX11*) is the founding member of a family of orphan *HOX* genes encoded in loci located outside the A, B, C and D paralog groups. In addition to *TLX1*, the *TLX* family of transcription regulators includes TLX2 and TLX3 (5) and is characterized by the presence of a threonine in the third helix of the homeodomain, which confers specific DNA binding properties. Like other *HOX* genes, *TLX* factors play important roles during development. Under normal conditions, *TLX1* expression is only detected during embryonic life in the mouse and is restricted to the branchial arches, the hindbrain and the splenic primordium (6, 7). Moreover, TLX1 plays a critical role of for spleen development and *TLX1* knock-out mice show complete agenesis of the spleen in the absence of other development alterations (8, 9).

Amongst T-ALL patients, TLX1 expressing tumors constitute a distinct molecular group characterized by a block in T-cell differentiation at the early cortical stage of T-cell development (2) and by a favorable prognosis (1, 2, 10). Moreover, *TLX1* induced leukemias represent a distinct oncogenic group with specific genetic alterations rarely found in non-TLX induced T-ALLs including the rearrangement of the *NUP214-ABL1* oncogene (11), deletion of the *PTPN2* phosphatase (12) and mutations in the *WT1* (13) and *PHF6* (14) tumor suppressor genes. However, until

recently, very little was known about the specific mechanisms that mediate T-cell transformation downstream of *TLX1*. Moreover, the generation of a mouse model of TLX1-induced T-ALL eluded the efforts of several groups over the last decade (15, 16). In this context, the generation of an LCK-*TLX1* transgenic mouse model that recapitulates the histological, transcriptional and genetic features of TLX1-induced T-ALL has provided a much needed tool for the analysis of the molecular and cellular mechanisms of TLX1-induced transformation (16). In these studies, LCK-*TLX1* transgenic mice showed accelerated mortality due to development of clonal T-cell acute lymphoblastic leukemia tumors occurring with an average latency of 6 months and a penetrance of more than 90% (16). Notably, almost identical phenotypes were observed in three different founder lines, ruling out a role for insertional mutations introduced during the generation of the transgene in the oncogenic process. TLX1-induced mouse leukemias showed clonal rearrangements of the *TCRB* locus (16) and were transplantable although with a low leukemia initiating cell content (Ferrando and De Keersmaeker unpublished results).

Mouse models of cancer have been instrumental in the analysis of genetic interactions between oncogenes and tumor suppressors and are increasingly being recognized as invaluable tools for the analysis of the basic mechanisms mediating oncogenic transformation, particularly with regard to tumor initiation and disease progression. Notably, analysis of T-cell differentiation in 3-6 week old *TLX1* transgenic mice showed that tumor development in these animals is preceded by a severe defect in thymocyte development characterized by a reduced thymus size, weight and cellularity. This T-cell development defect is mediated by increased apoptosis and a differentiation block at the earliest stages of thymocyte differentiation. Notably, gene expression profiling with oligonucleotide microarrays of TLX1-induced mouse T-ALLs compared with other genetic mouse models of T-ALL showed that TLX1 tumors have a distinct gene expression signature dominated by the downregulation of numerous transcripts. Cross speciess comparison of this

TLX1-associated mouse gene expression signature in human T-ALLs demonstrated a marked overlap of this transcriptional program with that of human tumors that express *TLX1* or the closely related *TLX3* transcription factor oncogene. These results suggest that TLX1 may be primarily working as a transcriptional repressor in T-ALL and that TLX1 and TLX3 may share a common gene expression program driving T-cell transformation. Moreover, the close relationship between the transcriptional programs of mouse and human TLX-induced T-ALLs underscore the relevance of TLX1-transgenic mice for the study of the basic mechanisms of *TLX*-induced transformation.

The 6 month long latency before the development leukemia in LCK-TLX1 transgenic mice (15, 16), and the observation that these tumors show clonal expression of the T-cell receptor beta, suggest that *TLX1* overexpression may not be sufficient for T-cell transformation, and that full leukemia development may depend on the acquisition of secondary co-operating mutations. The NOTCH1 signaling pathway plays a prominent role in T-ALL pathogenesis, with more than 50% of the patients harboring activating *NOTCH1* mutations (17). Consistently, about 12% of TLX1-induced tumors in mice showed activating mutations in the Notch1 gene (16). Extensive analysis of secondary chromosomal amplifications and deletions by array comparative genomic hybridization (aCGH) demonstrated focal deletions involving known T-ALL tumor suppressor genes such as *Pten, Trp53 and Cdkn2a/Cdkn2b* in TLX1-induced mouse tumors. In addition, the presence of a recurrent heterozygous deletion in mouse chromosome 12 encompassing the *Bcl11b* gene in three mouse *TLX1* tumors strongly suggested that *Bcl11b* could play a tumor suppressor role in T-ALL. The *Bcl11b* gene encodes a Kruppel-like zinc finger transcription factor with a critical role in the differentiation and survival of T-cell progenitors in the thymus(18, 19). BCL11B associates with the SIRT1 deacetylase (20) and has been implicated in transcriptional repression via its association with the NuRD nucleosome remodeling and histone deacetylase complex (21). Consistently, mutation analysis of *Bcl11b* in

mouse TLX1-induced tumors showed the presence of inactivating, heterozygous mutations in an additional 27% of the mouse tumors. In total, 47% of mouse TLX1 tumors analyzed showed heterozygous deletions or mutations targeting *Bcl11b*. Similarly, aCGH analysis of a panel of 69 human T-ALL patients identified 2 cases carrying focal heterozygous deletions targeting the *BCL11B* gene. In addition, 13% of T-ALL patient samples showed heterozygous *BCL11B* mutations. Overall, these results identified the *BCL11B* gene as a novel tumor suppressor gene recurrently deleted and mutated in T-ALL.

Analysis of *Bcl11b* knockout mice demonstrate that this transcription factor is strictly required for early T-cell development (18). Thus, *Bcl11b* homozygous knockout mice show a block in thymocyte development without defects in other blood lineages (18). Bcl11b seems to be critical for maintenance of T-cell identity so that loss of Bcl11b results in loss of T-cell lineage commitment and expression of transcriptional programs characteristic of non T-cell hematopoietic lineages. Moreover, *Bcl11b* haploinsufficiency results in marked decrease in thymocyte numbers and overt predisposition to lymphoma development (22). Notably, and of particular relevance for TLX1-induced transformation, TLX1 binds the promoter of the *BCL11B* gene and downregulates *BCL11B* expression (16). The model that emerges from these results is that aberrant expression of *TLX1* partially downregulates *BCL11B* during T-cell transformation, and that this negative transcriptional regulatory axis is fixed and reinforced by secondary genetic alterations in the *BCL11B* locus acquired during tumor progression (Fig. 1). Strikingly, *PHF6* and *WT1*, two additional tumor suppressors that are frequently mutated in *TLX1*-induced T-ALL (13, 14), are also TLX1 direct target genes (16), suggesting that this model may also apply to additional TLX1-target tumor suppressor genes (Fig. 1).

However, besides identifying focal areas of amplification and deletion, perhaps the most prominent cytogenetic alteration identified in TLX1-induced leukemias in mice is the presence of chromosomal gains and losses. Indeed, 78% of mouse TLX1 tumors

were aneuploid and showed a particularly high prevalence of trisomy 15. This intriguing observation suggested a close relationship between TLX1 overexpression and defects in the checkpoint machinery responsible for proper chromosome segregation during mitosis.

Early work on TLX1 had demonstrated that this transcription factor oncogene can interact with protein serine-threonine phosphatase 2A catalytic subunit (PP2AC) and protein phosphatase 1 (PP1C). Notably, inhibition of PP2A can regulate the cell cycle and TLX1 expression promoted progression to the M phase (23). Moreover, TLX1-expressing B-cells from IgHmu-HOX11Tg mice exhibited increased incidences of chromosome missegregation upon treatment with aneugenic agents and showed aberrant bypass of spindle checkpoint arrest (24). In agreement with these results, tumor cell lines derived from *TLX1* positive mouse T-ALLs showed a defective mitotic checkpoint (16). This defect in the control of progression through mitosis could be attributed to a direct effect of TLX1 on the mitotic machinery or alternatively result from secondary genetic alterations present in TLX1-induced tumors. Importantly, analysis of polyclonal thymocytes from preleukemic TLX1-transgenic mice demonstrated the presence of impaired mitotic checkpoint linking the expression of TLX1 with the induction of aneuploidy at the earliest stages of T-cell transformation. Finally, integrative analysis of gene expression signatures with Chip-chip data on direct TLX1 targets showed a marked enrichment of mitotic genes directly controlled by TLX1 among the genes downregulated in preleukemic TLX1-expressing thymocytes compared with cells from normal littermate controls (Fig. 1)(16). One of these genes was *CHEK1,* a crucial player in proper function of the cell cycle machinery and the mitotic checkpoint (16).

These results reveal a previously unrecognized role for an oncogenic transcription factor in mitotic checkpoint deregulation in T-ALL pathogenesis. It remains to be determined whether other oncogenes and tumor suppressors in T-ALL are cooperating with TLX1 in deregulation of cellular checkpoints. MYC, an important

downstream player of NOTCH1 in T-ALL (25), has been shown to directly regulate crucial factors in the mitotic checkpoint machinery (26). In addition, the *PTEN* tumor suppressor gene is inactivated in 17% of T-ALL cases (27), and PTEN loss induces defects in multiple cell cycle checkpoints, including the mitotic spindle checkpoint (28, 29). Still, it remains to be determined whether oncogenic transcription factors in other cancer types are stressing the cellular mitotic checkpoint machinery by similar mechanisms as TLX1 in the context of T-ALL.

CLINICAL-TRANSLATIONAL ADVANCES

T-ALL patients often have a large tumor burden with mediastinal masses, very high circulating blast cell counts and infiltration of the central nervous system at the time of diagnosis (30). In the early days of combination chemotherapy for ALL, T-ALL was associated with very poor prognosis, with remission durations of only 10 months or less and cure rates lower than 10% (31). However, after the introduction of intensified chemotherapy schemes, a remarkable improvement was observed in the prognosis of children and adolescents with this disease, so that 5-year relapse-free survival rates are now over 75% (32). Similarly, wider use of intensified therapy has resulted in advances in the treatment of adults with T-ALL, with cure rates now exceeding 50% (33). Our current understanding of the molecular basis of T-cell malignancies has emerged largely from analysis of recurrent chromosomal translocations, which typically juxtapose strong promoter elements to developmentally important transcription factor genes (34, 35). These translocations induce aberrant expression of oncogenic transcriptional regulators in developing T-cells. This group of transcription factors includes: (i) basic helix-loop-helix (bHLH) proteins encoded by *TAL1, TAL2, LYL1* and *BHLHB1* (36-40) (41); (ii) LIM-only domain (LMO) proteins encoded by *LMO1* and *LMO2* (42-45)*;* (iii) products of the orphan homeobox genes *TLX1* (46-49) and *TLX3* (2, 50); and mutated or truncated forms of *NOTCH1* encoding constitutively activated forms of the NOTCH1 receptor (17, 51). In addition, rare cases show rearrangements and mutations generating

oncogenic tyrosine kinases such as NUP214-ABL1 and JAK1 (11, 52). From a therapeutic point of view, administration of small molecule kinase inhibitors may offer an opportunity for molecularly tailored therapies for cases expressing oncogenic kinases. In addition, gamma-secretase inhibitors that block NOTCH1 signaling looked very promising to treat T-ALL. However, the first clinical trials testing gamma-secretase inhibitor monotherapy demonstrated serious toxicity and inefficacy of such treatment for T-ALL, and evidence has been collected that combining gamma-secretase inhibitors with glucocorticoids might increase the therapeutic potential of gamma-secretase inhibitors in T-ALL (53).

Over the last decade, several laboratories have characterized the mechanisms that mediate the transforming effects of activated NOTCH receptors and bHLH factors (54-57), however, much less is known about the genetic programs operating downstream of TLX1. This TLX family member yields leukemias with a good prognostic outcome (1, 2, 10), which was completely unexplained seen our ignorance on the oncogenic mechanisms by which TLX1 is acting in T-ALL. After the discovery that TLX1 induces malfunction of the cellular mitotic checkpoint (16), it is tempting to speculate that this defective mitotic checkpoint in *TLX1* tumors could indeed be related to increased sensitivity to chemotherapy drugs inducing DNA damage and/or targeting the mitotic spindle.

The absence of a role for TLX1 during adult life and its restricted expression in thymocytes carrying t(10;14)(q24;q11) suggest that TLX1 might be an attractive target for molecularly tailored therapy. Transcription factors function in multiprotein complexes dependent on extended protein-protein interactions and have been traditionally considered poor targets for drug development. However, detailed structural data, improvements in small molecule design and peptide engineering have facilitated the development of specific inhibitors of the BCL6 and NOTCH1 transcription factor oncogenes (58-61). Identification of the protein complex mediating TLX1 function in T-ALL and resolving the crystal structure of this

homeobox gene in complex with DNA and essential coregulatory proteins may eventually facilitate the generation of specific TLX1 inhibitors for the treatment of T-ALL. Finally, the implementation of shRNA screens directed to identify genes and pathways whose inactivation results in specific ablation of TLX1-expressing cells may uncover new targets for the treatment of TLX1-induced leukemias. In this context, mouse models of TLX1-induced T-ALL will be instrumental for the validation and preclinical testing of anti-TLX1 therapies in their translation to the clinic.

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FIGURE 1. T-ALL induction by TLX1.

The t(10;14)(q24;q11) chromosomal translocation induces results from the aberrant rearrangement of the *TCRA-D* locus with the *TLX1* gene and results in high levels of expression of *TLX1* transcripts in T-cell progenitor cells. TLX1 binds to regulatory sequences to repress the expression of target genes. TLX1 targets include mitotic checkpoint regulatory genes such as *CHEK1*, *BUB1* and *BRCA2* and TLX1 expression results in malfunction of the mitotic checkpoint machinery facilitating chromosomal missegregation and aneuploidy. Additional TLX1 target genes include the *BCL11B*, *WT1* and *PHF6*, tumor suppressor genes which are frequently mutated in TLX1-induced leukemia. In addition, TLX1 regulates expression of numerous targets, some of which mediate additional oncogenic effects such as block of T-cell differentiation. The aneuploidy promoting action of TLX1, its role in blocking T-cell maturation and the acquisition of secondary mutations eventually lead to transformation of T-cell progenitors into T-ALL.

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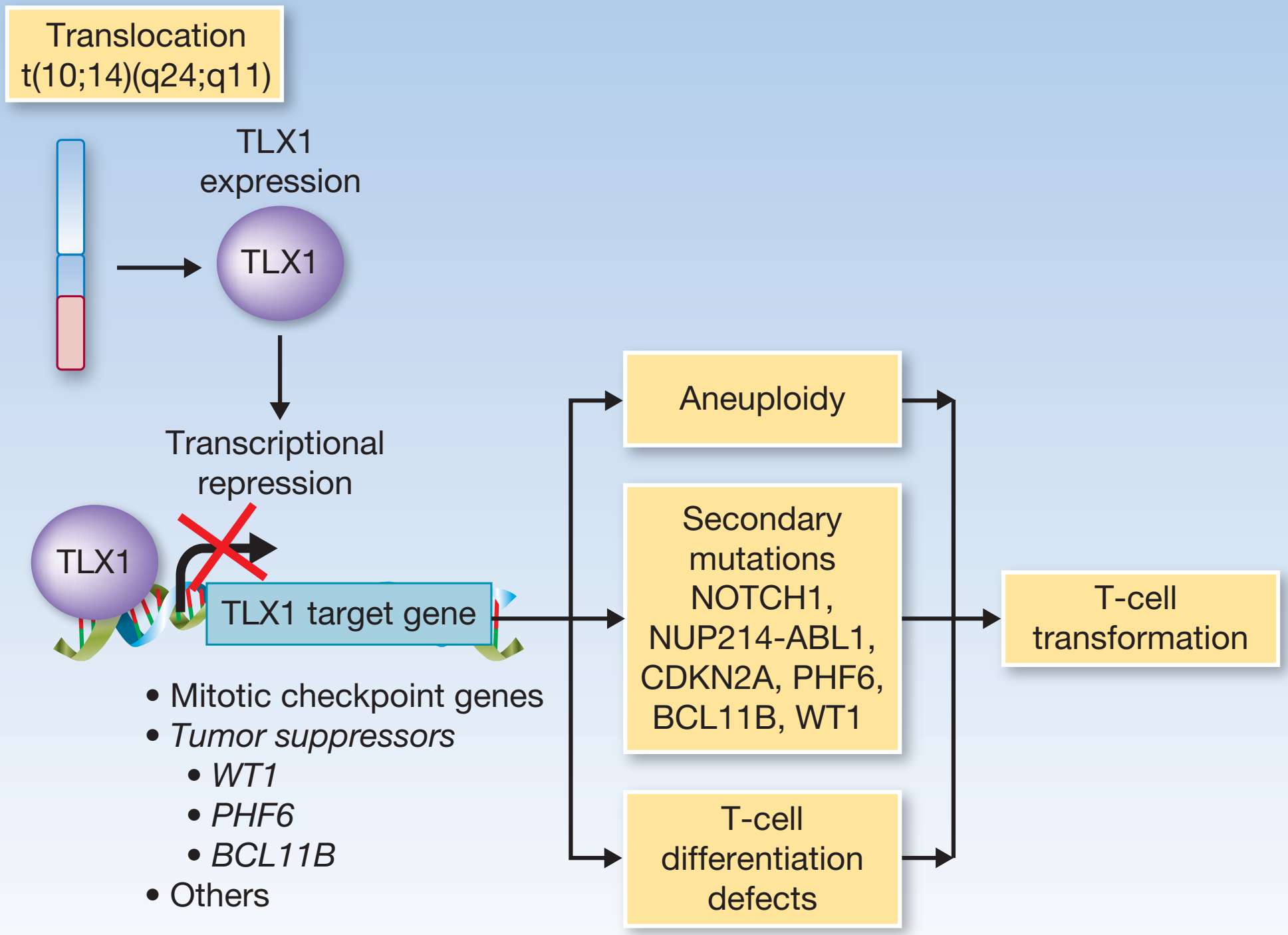

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