Original Articles

LMO2 expression reflects the different stages of blast maturation and genetic features in B-cell acute lymphoblastic leukemia and predicts clinical outcome

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ABSTRACT

Background

LMO2 is highly expressed at the most immature stages of lymphopoiesis. In T-lymphocytes, aberrant *LMO2* expression beyond those stages leads to T-cell acute lymphoblastic leukemia, while in B cells *LMO2* is also expressed in germinal center lymphocytes and diffuse large B-cell lymphomas, where it predicts better clinical outcome. The implication of *LMO2* in B-cell acute lymphoblastic leukemia must still be explored.

Design and Methods

We measured *LMO2* expression by real time RT-PCR in 247 acute lymphoblastic leukemia patient samples with cytogenetic data (144 of them also with survival and immunophenotypical data) and in normal hematopoietic and lymphoid cells.

Results

B-cell acute lymphoblastic leukemia cases expressed variable levels of *LMO2* depending on immunophenotypical and cytogenetic features. Thus, the most immature subtype, pro-B cells, displayed three-fold higher *LMO2* expression than pre-B cells, common-CD10+ or mature subtypes. Additionally, cases with *TEL-AML1* or *MLL* rearrangements exhibited two-fold higher *LMO2* expression compared to cases with *BCR-ABL* rearrangements or hyperdyploid karyotype. Clinically, high *LMO2* expression correlated with better overall survival in adult patients (5-year survival rate 64.8% (42.5%-87.1%) *vs.* 25.8% (10.9%-40.7%), P= 0.001) and constituted a favorable independent prognostic factor in B-ALL with normal karyotype: 5-year survival rate 80.3% (66.4%-94.2%) *vs.* 63.0% (46.1%-79.9%) (P= 0.043).

Conclusions

Our data indicate that *LMO2* expression depends on the molecular features and the differentiation stage of B-cell acute lymphoblastic leukemia cells. Furthermore, assessment of *LMO2* expression in adult patients with a normal karyotype, a group which lacks molecular prognostic factors, could be of clinical relevance.

Key words: acute leukemia, B lymphocytes, gene expression, prognostic factor.

Introduction

The Lim Domain Only 2 gene (*LMO2*, also termed *RBTN2* and *TTG2*) encodes a cystein-rich LIM domain-containing transcription factor that is required for complete hematopoiesis in mice.[1] While *LMO2* is expressed at various levels in virtually every tissue in fetal and adult life, its expression in hematopoietic cells is tightly regulated and varies at different ontogeny stages of maturation.[2] *LMO2* was originally identified through its involvement in recurrent chromosomal translocations in T-cell acute lymphoblastic leukemia (T-ALL). Indeed, constitutive activation of *LMO2* in T cells by juxtaposition with the T-cell receptor (TCR) gene loci through t(11;14)(p13;q11) or t(7;11)(q35;p13), or by the cryptic deletion del(11)(p12p13), is characteristic of T-ALL.[3,4] In normal T-cell development, *LMO2* is expressed in immature CD4/CD8 double negative thymocytes, but its expression is down-regulated during T-cell maturation and is absent in mature T cells.[5] Ectopic expression of *LMO2* in the T-cell lineage in transgenic mouse models leads to clonal expansion of T cells, eventually generating human-like T-ALL.[6] Moreover, during gene therapy of patients with X-linked severe combined immunodeficiency (X-SCID), retroviral insertion in the proximity of the *LMO2* gene resulted in the overexpression of this gene and development of T-ALL in 3 children.[7] These data demonstrate that *LMO2* functions as an oncogene in this leukemia.

In normal B-cell differentiation and maturation, *LMO2* is expressed mainly at two stages: at early lymphopoiesis within the bone marrow and in germinal centers (GC) of secondary lymphoid organs. *LMO2* expression is also found in B-cell lymphomas derived from GC lymphocytes, including follicular, Burkitt's and diffuse large B-cell (DLBCL) lymphomas, as well as in lymphocyte-predominant Hodgkin's lymphoma.[2] Remarkably, *LMO2* expression is an independent prognostic factor of survival in patients with DLBCL treated with anthracycline-based chemotherapy with and without rituximab,[8] as well as in chronic myeloid leukemia[9] and pancreatic cancer.[10] While marked advances have been made in establishing the significance and function of *LMO2* in T-ALL and B-cell lymphomas, its role in B-cell acute lymphoblastic leukemia (B-ALL) has not been investigated.

B-ALL comprises cytogenetically distinct subgroups defined by specific recurrent chromosomal translocations or by the presence of hyperdiploid and hypodiploid karyotypes. Clinically, the distinction between these genetic subgroups is important for prognosis and selection of optimal therapeutic options. In addition to cytogenetic analysis, the study of the immunophenotype of the blasts is also important in the diagnosis of B-ALL, and the distinction between T-, mature B- and precursor B-phenotypes affects therapeutic decision making.[11]

This report assesses *LMO2* expression in a series of patients with B-ALL. Our data suggest that, in contrast to T-ALL, *LMO2* expression reflects the developmental stage in which the blasts are arrested rather than being an oncogenic event in this disease. Furthermore, we show that *LMO2* expression correlates with survival of B-ALL patients, being an independent prognostic factor in the subgroup with normal karyotype, particularly among patients over 15 years of age.

Design and Methods

Patients' samples and cell lines

LMO2 expression was assessed in a cohort of 247 patients with acute lymphoblastic leukemia (ALL) who were treated according to the PETHEMA (Spanish Group for the Study and Therapy of Haematological Malignances) protocols at the Departments of Haematology of the Clínica Universidad de Navarra (Pamplona), Hospital Reina Sofía (Cordoba) and other national institutions belonging to the PETHEMA group.[12,13] Full clinical and laboratory data were available for 39 T-ALL and 145 B-ALL patients (one of them lacking survival data) with a median age of 11.5 years (range 0.5-87 years), including 100 children under 15 years of age (median 5.5 years, range 0.5-14) and 84 patients over 15 years of age (mean age 34.5, range 15-87). Sample collection was approved by the individual institutional review boards of the participating institutions and informed consent was obtained from all adult patients and children's parents or legal guardians.

Cytogenetic and molecular analyses were performed for all samples (*Online Supplementary Design and Methods*). We found 32 cases (15%) with *TEL-AML1* (*ETV6-RUNX1*) gene rearrangement, 41 cases (20%) with *BCR-ABL* rearrangement, 16 cases (8%) with rearrangements involving the *MLL* gene, 9 cases (4%) with hyperdiploid karyotype (more than 50 chromosomes), 21 cases (10%) with miscellaneous abnormalities and 89 cases (43%) with normal karyotype. Detailed information on the number of cases with cytogenetic, immunophenotypical and survival data available is provided in the *Online Supplementary Table S1 and Figure S1*. The clinical and immunophenotypical characteristics of the B-ALL patient samples are shown in Table 1. Methods for the sorting of the normal lymphocyte subpopulations are available in the *Online Supplementary Appendix and Table S2*, along with the cell lines included in this study.

Quantitative real time PCR (Q-RT-PCR)

All patient samples were taken at initial diagnosis and come from bone marrow aspirates. Only samples with more than 80% blasts were included in this study. Total RNA from patients' mononuclear bone marrow cells was extracted with Ultraspec RNA isolation system (Biotecx). RNA quantity and quality were assessed by spectrophotometric measurements with NanoDrop® ND-1000 Spectrophotometer. Reverse transcription and Q-PCR were performed as previously described[14] using specific TaqMan probes and 10x LightCycler TaqMan Master (Roche Applied Science). *LMO2* expression was normalized to *ABL* expression as recommended by the Europe Against Cancer group for leukemic samples.[15] This group reported that *ABL* expression remained constant not only in normal blood/bone marrow samples but also in leukemic samples with different genetic alterations, including cases with *BCR-ABL* rearrangement. We confirmed that *ABL* expression remained constant among our samples with different genetic alterations and in the normal karyotype subgroup (*P*= 0.761) (*Online Supplementary Figure S2*). The following primers were used for PCR: forward *LMO2* primer 5'-GGCGGCGCCTC-TACTACA-3', reverse *LMO2* primer 5'-CCAAAAAGCCTGA-GATAGTCTCT-3', TaqMan LMO2 probe 5'-CTGGGCCG-GAAGCTCTGCC-3'. Forward *ABL* primer 5'-TGGAGATAA-CACTCTAAGCATAACTAAAGGT-3', reverse *ABL* primer 5'-GATGTAGTTGCTTGGGACCCA-3', TaqMan *ABL* probe 5'-CCATTTTTGGTTTGGGCTTCACACCATT-3'.

Total RNA from cell lines and normal lymphocyte subpopulations was extracted using TRIzol reagent (Invitrogen). cDNA synthesis was performed with M-MLV reverse transcriptase (Invitrogen) and Q-PCR was performed in the 7300 Real Time

Table 1. Clinical parameters in B-ALL patients.

Characteristic	Total	High *LMO2* expression (N=73)	Low *LMO2* expression (N=72)	*P* value
Age (y)				*P*= 0.101
Median	11	8	18	
Range	0.5-76	0.5-73	1-76	
Age group				*P*= 0.081
Childhood (<15 y)	81	46	35	
Adult (≥15 y)	64	27	37	
Gender				*P*= 0.564
Male	78	41	37	
Female	67	32	35	
Hyperleukocytosis				*P*= 0.781
Present	38	20	18	
Absent	104	52	52	
Immunophenotype				*P*= 0.001*
Pro-B	35	28	7	
Common-CD10+	94	40	54	
Pre-B	12	4	8	
Mature	4	1	3	
BCR-ABL				*P*= 0.001*
Present	22	4	18	
Absent	123	69	54	
TEL-AML1				*P*= 0.797
Present	25	12	13	
Absent	120	61	59	

*Three patients lacked data on hyperleukocytosis. The P values correspond to Pearson's χ² test except for the parameter "Age" that was compared with the Mann-Whitney U test. *Significant P values.*

Table 2. Factors associated with prolonged overall survival of B-ALL patients.

Variable	Log rank *P* value	Multivariate Cox's regression *P* value
Categorical *LMO2* expression	0.004*	0.068
Age group	<0.001*	0.003*
BCR-ABL	<0.001*	<0.001*
TEL-AML1	0.001*	0.958
Hyperleukocytosis	0.025*	0.340
Gender	0.340	N.A.

*Significant P values.

given as median and range. Due to the lack of fit to the normal distribution of *LMO2* expression values, tested by Kolmogorov-Smirnov and Shapiro-Wilk tests, non-parametric comparisons were performed: Mann-Whitney U test for comparisons of two groups or Kruskal-Wallis test for comparisons of more than two groups. Holms-Bonferroni correction was applied in cases of multiple comparisons with the Mann-Whitney U test. Correlation between continuous variables was assessed by Spearman's correlation test. Differences were considered significant when $P< 0.05$.

Results

LMO2 expression changes during B-cell lymphopoiesis and at distinct differentiation stages

LMO2 expression was measured by Q-RT-PCR in different normal hematopoietic and B-cell subpopulations representing distinct differentiation stages. *LMO2* expression was highest at the earliest stages of bone marrow hematopoiesis, decreasing gradually from hematopoietic stem cells (HSCs) to the pro-B cell stage: median 38.23 (range 35.16-41.30) in HSCs, 19.08 (11.72-26.44) in early B cells, and 14.59 (8.04-21.14) in pro-B cells. After the pro-B stage, a sharp approximately 8-fold decrease in *LMO2* expression was observed: 1.71 (1.10-2.32) in pre-B cells and 1.90 (1.55-2.25) in immature B cells. In secondary lymphoid organs, *LMO2* expression was increased at the GC B-cell stage [13.81 (13.29-19.54)] compared to naïve B cells [5.67 (5.06-7.78)] and memory B cells [3.26 (3.16-3.95)] (Figure 1).

Comparison of LMO2 expression in B-ALL, T-ALL, DLBCL and B-cell precursors

Similar levels of *LMO2* expression were observed in primary B-ALL samples [2.13 (0.21-20.04)] and B-ALL cell lines [1.63 (0.21-17.54)] (P=0.986) as well as in the pre-B cell precursor subpopulation [1.71 (1.10-2.32)] (P=0.566) (Figure 1). Next, we compared *LMO2* expression between cell lines of different origins. *LMO2* expression tended to be lower in T-ALL [0.71 (0.00-3.11)] and GCB-DLBCL [1.45 (0.03-4.41)] cell lines compared to B-ALL cell lines but the difference did not reach statistical significance (P=0.151 and P=0.080, respectively) (Figure 1). As expected, *LMO2* expression in ABC-DLBCL cell lines [0.02 (0.00-1.41)] was lower compared to B-ALL and GCB-DLBCL cell lines (P=0.018 and P=0.035, respectively).

Immunophenotypical and cytogenetic subgroups of B-ALL exhibit variable levels of LMO2 expression

LMO2 expression was significantly higher in B-ALL patients than in T-ALL patients [1.66 (0.04-10.90) *vs.* 0.70

PCR System with Universal PCR Master Mix (Applied Biosystems). Hs 00277106_m1 (Applied Biosystems) was the probe for *LMO2* and Hs 99999906_m1 for *PGK1*, selected as reference gene due to its good performance in our experience with lymphoid samples and cell lines.[16] *LMO2* expression was also assessed using these probes in 54 B-ALL patients to compare them with normal lymphocytes and cell lines.

When using either *ABL* or *PGK1* as housekeeping genes, *LMO2* expression was calculated as a ratio between the amount of *LMO2* referred to a standard curve of cDNA and the amount of the reference gene referred to a standard curve of the same cDNA. Correlation between the *LMO2* expression results obtained using *PGK1* or *ABL* as housekeeping genes was highly significant (P=0.003, Spearman's Rho= 0.393).

Statistical analysis

The SPSS 15.0 software was used for statistical analyses. Kaplan-Meier curves and the log rank test were used for univariate survival analysis, for which *LMO2* expression was categorized using its mean expression among all B-ALL patients as threshold value (2.29). Overall survival (OS) was defined as the time interval between the date of diagnosis and the date of death or last follow up. Event-free survival (EFS) was defined as the time interval from the date of initial diagnosis to the date of disease progression, death from any cause or last follow-up evaluation. The relevant clinical variables of these patients were also tested for their possible correlation with survival by the log rank test (Table 2), and a multivariate Cox's regression assay was performed including all variables with $P< 0.05$. The possible association between nominal variables and categorical *LMO2* expression was tested by Pearson's χ² test. *LMO2* expression was used as a continuous variable for the remaining comparisons. Representative values are

(0.04-10.00), *P*=0.010] (Figure 2A). Cytogenetic and molecular data on the B-ALL cases allowed the distinction of six specific genetic subgroups with hyperdiploidy, *BCR-ABL* rearrangement, *TEL-AML1* rearrangement, translocations involving the *MLL* locus, normal karyotype and cases with miscellaneous alterations. B-ALL cases with *BCR-ABL* rearrangement or hyperdiploid karyotype showed the lowest *LMO2* expression values [0.84 (0.00-3.30) and 0.80 (0.10-7.80) respectively], while all the remaining cases, including those with *MLL* translocations [1.92 (0.10-10.90)] or *TEL-AML1* rearrangement [1.91 (0.10-5.40)] exhibited higher levels of *LMO2* expression (*P*=0.001) (Figure 2B).

B-ALL patient samples were also subclassified in four different immunophenotypical subgroups (pro-B, common-CD10+, pre-B and mature). Among these subgroups, pro-B cell ALL showed the highest *LMO2* expression [4.49 (0.90-10.90)] while B-ALL with common-CD10+ immunophenotype [1.96 (0.10-7.80)], pre-B cell ALL [1.42 (0.20-5.70)] and mature B-ALL [1.10 (0.50-2.70)] had lower expression (*P*<0.001) (Figure 2C). Notably, the difference in *LMO2* expression between the pro-B and pre-B leukemia immunophenotypes resembled the decrease in *LMO2* expression observed upon transition of pro-B to pre-B cells in normal bone marrow (Figure 1).

LMO2 *expression is associated with survival in patients with B-ALL*

Previous studies demonstrated that increased *LMO2* expression correlates with better survival in DLBCL, as well as in chronic myeloid leukemia and pancreatic cancer. Therefore, we examined the possible correlation between *LMO2* RNA expression and patient clinical outcome in ALL. We divided the patients in two subgroups with high or low levels of *LMO2* expression using the mean *LMO2* expression value of the whole cohort of B-ALL patients
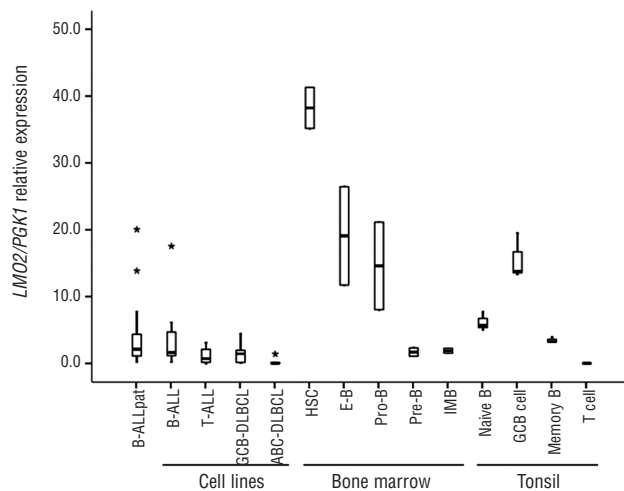

Figure 1. *LMO2* expression during B-cell differentiation and in B-lymphoid malignancies. *LMO2* expression was measured by Q-RT-PCR. The bar inside the boxes represents the median value, the limits of the box the interquartile range, the whiskers the range of expected values and (*) are outlier values. B-ALL: B-cell acute lymphoblastic leukemia, pat: patient samples, T-ALL: T-cell acute lymphoblastic leukemia, ABC-DLBCL: activated B-cell like diffuse large B-cell lymphoma, GCB-DLBCL: germinal center B-cell like diffuse large B-cell lymphoma, HSC: hematopoietic stem cell, E-B: early B cell, IMB: immature B cell, GCB=germinal center B cell.

(2.29) as cut-off value. The median overall survival (OS) was not reached in any of the two subgroups. The OS rate was significantly higher in B-ALL patients with high levels of *LMO2* expression compared to patients with low levels of *LMO2* expression: the 5-year cumulative survival rate with 95% CI was 77.2%(66.8%-87.6%) *vs.* 56.1%(44.1%-68.1%); *P*=0.004 (Figure 3A). To determine whether *LMO2* expression is an independent predictor for OS in B-ALL, we initially examined whether other known clinical and laboratory prognostic factors also correlated with OS by log rank test. In univariate analysis, age under 15 years at diagnosis (childhood B-ALL) and the presence of *TEL-AML1* gene rearrangement correlated with favorable prognosis, whereas the presence of hyperleukocytosis (leukocyte count > 50,000/mm³) or *BCR-ABL* gene fusion were associated with shorter OS (Table 2). Analyses of the possible correlation of *MLL* gene rearrangements or hyperdiploid karyotypes with survival in our cohort of patients were not performed because of the small number of cases in these subgroups, which precluded any robust statistical conclusion.

A multivariate Cox's regression analysis including age group, hyperleukocytosis, presence of *BCR-ABL* or *TEL-AML1* gene rearrangements and subgrouping based on *LMO2* expression was performed. Only age over 15 years and the presence of *BCR-ABL* gene rearrangement were independently associated with worse OS in B-ALL (*P*=0.003 and *P*<0.001, respectively). In contrast, *LMO2* expression was not independently correlated with OS, suggesting its association with age and/or the presence of *BCR-ABL* rearrangement. By using Pearson's χ^2 test, a highly significant association between the presence of *BCR-ABL* rearrangement and low *LMO2* expression was found (*P*=0.001). As age group is one of the main prognostic factors in our cohort of patients, and the treatment protocols are more aggressive and effective in the age group under 15 years, we performed a separate study on the association between *LMO2* expression and survival for each age group. We found that in adult B-ALL cases high *LMO2* expression is a predictor of better outcome (5-year survival rate 64.8% (42.5-87.1%) *vs.* 25.8% (10.9-40.7%), *P*=0.001) (Figure 3B), while in patients under 15 years of age there is no difference in mortality between the patients with high or low *LMO2* expression (*P*=0.673), probably due to the low number of events among patients in this age group (5-year survival rate 84.8% (76.4-93.2%) *vs.* 42.7% (28.8-56.6%) in adults). As far as event-free survival (EFS) is concerned, patients older than 15 years with high *LMO2* expression displayed better survival rates than those with low *LMO2* expression (5-year survival rate 76.9% (59.1-94.7%) *vs.* 35.2% (9.9-60.5%), *P*=0.009) (Figure 3C).

LMO2 *expression is an independent prognostic factor among the B-ALL cases with normal karyotype*

In our series, the subgroup with normal karyotype included the highest number of cases. The presence of a normal karyotype did not correlate with OS (*P*=0.101). Remarkably, in this subgroup of patients high *LMO2* expression was associated with better OS: the 5-year survival rate was 80.3% (66.4-94.2%) *vs.* 63.0% (46.1-79.9%) (*P*=0.043) (Figure 3D). Moreover, when multivariate Cox's regression analysis was applied, testing the variables *LMO2* expression levels, age group and hyperleukocytosis, *LMO2* expression and age group remained independ-
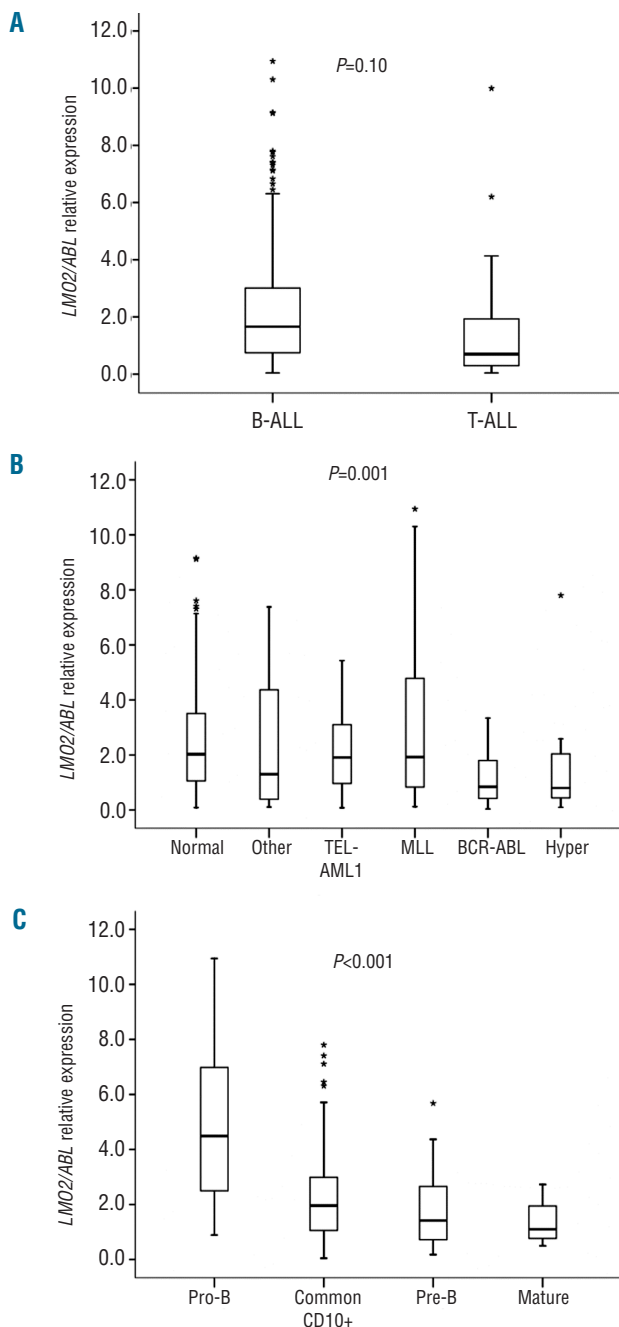
A

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C

expressed in this disease, as previously proposed.[2,17] Most B-ALL cases exhibited *LMO2* expression levels similar to those measured in non-transformed pre-B cells, and even in the cases with the highest *LMO2* expression these levels did not exceed those found in non-transformed pro-B cells. As bone marrow B-cell precursors (both pro-B and pre-B cells) are immunophenotypically very similar to the blasts of the majority of B-ALL cases, the range of *LMO2* expression found in B-ALL probably reflects the stage at which these blasts undergo differentiation arrest. In this regard, measurement of the expression of *LMO2* in a B-ALL sample might help to assess the grade of differentiation of the malignant cells. Furthermore, *LMO2* expression was associated with different cytogenetic subtypes of B-ALL, being particularly low in *BCR-ABL* and hyperdiploid cases. Overall, *LMO2* RNA expression levels in B-ALL seem to reflect the molecular and phenotypical characteristics of the leukemic cells.

Despite the clear oncogenic role of *LMO2* in T-ALL, this is probably not the case in B-ALL. No translocations of this gene have been reported in this disease, and other genetic alterations involving the *LMO2* locus are extremely rare among B-ALL cases. For instance, genome-wide analysis of 192 B-ALL samples using SNP arrays detected a deletion of 155 kb upstream of the *LMO2* gene locus in only one case, in contrast to the 8% of T-ALL cases that showed this alteration.[18] Furthermore, among 226 B-ALL cases analyzed in our institution by conventional cytogenetics, 154 presented chromosomal abnormalities, but none showed alterations in 11p13 (*MJ Calasanz, unpublished data, 2010*). Moreover, in 3 out of 10 X-SCID patients treated with retrovirus-mediated gamma(c) gene transfer into autologous CD34+ bone marrow cells, integration of the retrovirus vector in the *LMO2* gene promoter induced aberrant *LMO2* expression that led exclusively to T-ALL.[19] Interestingly, while mature T cells repress *LMO2* expression after their differentiation in the thymus,[5] B cells greatly induce *LMO2* expression during the immune response in the GC reaction, reaching levels similar to that of pro-B cells (Figure 1). This observation may suggest that elevated *LMO2* expression in B cells is not necessarily an oncogenic event *per se*. Thus, all these data suggest that in contrast to T-ALL, *LMO2* does not act as an oncogene in B-ALL cells. On the other hand, a recent report has described that in B-ALL cells with t(17;19) (found in only 1% of B-ALL patients[20]) the resulting fusion protein E2A/HLF induces the transcription of *LMO2*, and upon *LMO2* silencing with shRNA the apoptotic rate increases by almost 10%.[21] Nevertheless, this effect on apoptosis is modest and it is possible that the oncogenic potential of E2A/HLF depends on other transcription targets. Furthermore, mice transgenic for E2A/HLF fusion protein develop lymphoid

ent prognostic factors (*P*=0.045 and *P*=0.007, respectively).

When we performed a log rank test separately in the two age groups, we found that higher *LMO2* expression remained a predictor of better outcome only in the patients over 15 years of age: 5-year survival rate 70.0% (36.7-103.3%) *vs.* 34.3% (9.4-59.2%), *P*=0.008 (Figure 3E). We did not find any statistical association of EFS with *LMO2* expression in the group of patients with normal karyotype and over 15 years of age.

Discussion

The role of *LMO2* as oncogene in T-ALL[6] and, conversely, as favorable prognostic factor in DLBCL,[8] CML[9] and pancreatic cancer,[10] led us to evaluate the practically unexplored role of *LMO2* in B-ALL. We found that *LMO2* is
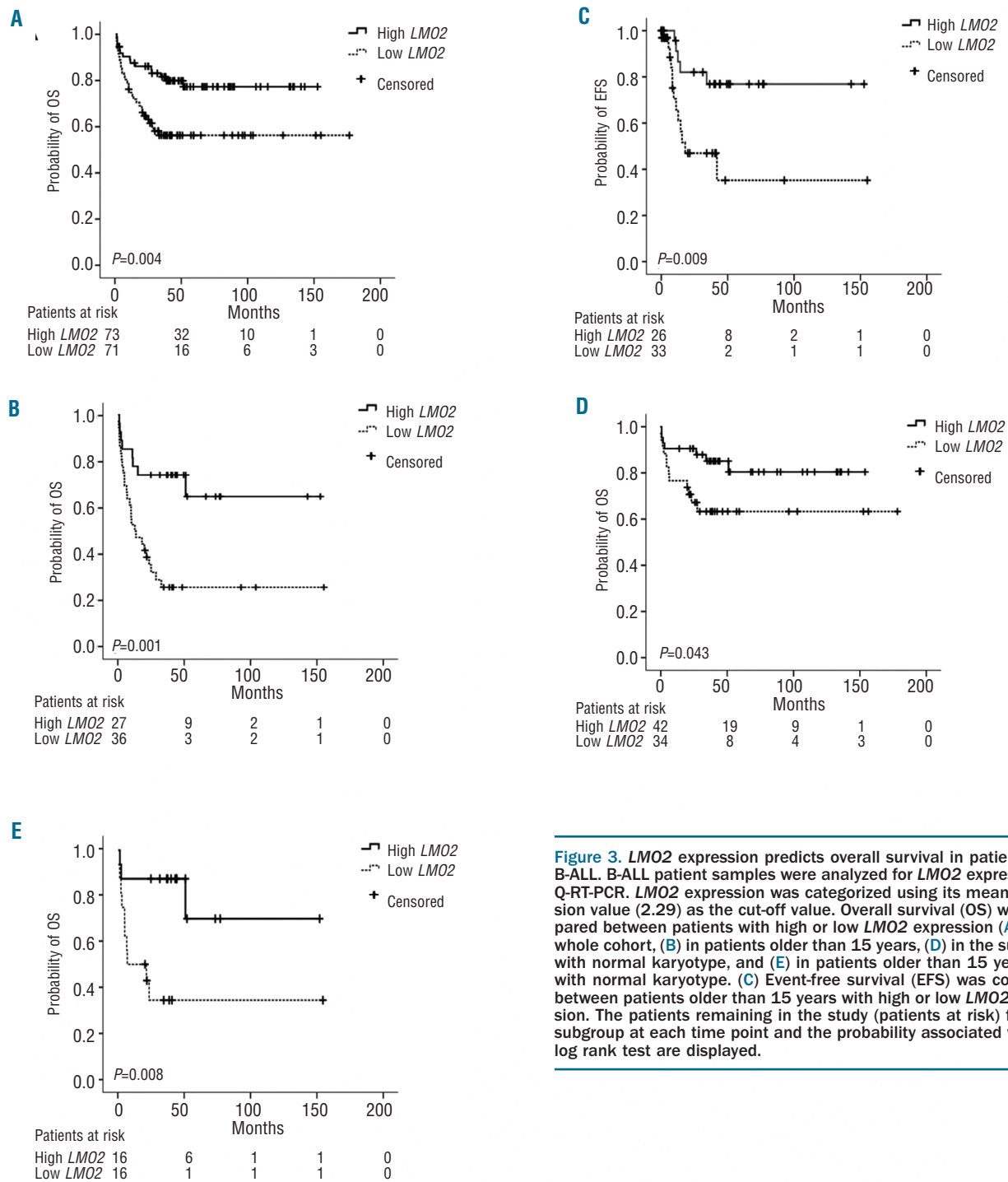
Figure 3. *LMO2* expression predicts overall survival in patients with B-ALL. B-ALL patient samples were analyzed for *LMO2* expression by Q-RT-PCR. *LMO2* expression was categorized using its mean expression value (2.29) as the cut-off value. Overall survival (OS) was compared between patients with high or low *LMO2* expression (A) in the whole cohort, (B) in patients older than 15 years, (D) in the subgroup with normal karyotype, and (E) in patients older than 15 years and with normal karyotype. (C) Event-free survival (EFS) was compared between patients older than 15 years with high or low *LMO2* expression. The patients remaining in the study (patients at risk) for each subgroup at each time point and the probability associated with the log rank test are displayed.

malignancies with T-cell phenotype, and only very rarely with B-cell precursor phenotype.[22,23]

Remarkably, we have found that high *LMO2* expression correlates with longer overall survival (OS) in patients with B-ALL, in agreement with reports in DLBCL, CML and pancreatic cancer. Part of this prognostic association in our cohort of B-ALL patients may be attributed to the low levels of *LMO2* expression found in the subgroup of cases with *BCR-ABL* rearrangement, a well-known adverse prognostic factor.[24,25] Interestingly, multivariate regression analysis identified high *LMO2* expression as an independ-

ent favorable prognostic factor for OS in patients with normal karyotype, particularly in patients over 15 years of age, for which treatment protocols are not as intense and effective as those used in younger patients. Because this heterogeneous subgroup lacks known cytogenetic or molecular markers that may predict survival, evaluation of *LMO2* expression in these patients may provide valuable additional information in terms of risk stratification and therapeutic tailoring.

In terms of event-free survival (EFS), high *LMO2* expression correlated with better outcome in B-ALL patients

over 15 years of age, agreeing with its usefulness as prognostic factor in this subgroup. We could not find any association between *LMO2* expression and EFS in the subgroup of patients over 15 years of age and with normal karyotype, probably due to the low number of relapses in this subgroup (event-free survival data was only available in 5 of the 28 patients in this subgroup).

It was recently reported that LMO2 protein expression assessed by immunohistochemistry in 22 cases of B-ALL did not predict survival in that patient cohort.[17] The low number of B-ALL cases enrolled in this study, as well as the lack of stratification by immunophenotypical or cytogenetic characteristics of the blasts are probably the reasons why this study failed to find statistical correlation between LMO2 expression and survival in B-ALL.

In summary, B-ALL cases express variable levels of *LMO2* that are associated with the immunophenotypical and molecular features of this disease and correlate with OS in our cohort of patients. Measurement of *LMO2* expression should be of particular interest in B-ALL adult cases with normal karyotype, in which high *LMO2* expression is an independent favorable prognostic factor for OS that could have clinical and therapeutic relevance.

Authorship and Disclosures

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