**ISSN** 1672-9145 **Acta Biochimica et Biophysica Sinica** 2005, **37**(3): 173–180 **CN** 31-1940/Q

# Alterations of Lymphoid Enhancer Factor-1 Isoform Expression in Solid Tumors and Acute Leukemias

Wenbing WANG[1], Ping JI[2]*, Björn STEFFEN[2], Ralf METZGER[3], Paul M. SCHNEIDER[3], Hartmut HALFTER[4], Mark SCHRADER[5], Wolfgang E. BERDEL[2], Hubert SERVE[2], and Carsten MÜLLER-TIDOW[2]*

[1]*Institute of Life Sciences, Jiangsu University, Zhenjiang 212013, China;*
[2]*Hematology and Oncology, and* [3]*Neurology, Department of Medicine, University of Münster, D-48129 Münster, Germany;*
[4]*Department of Visceral and Vascular Surgery, University of Cologne, D-50931 Cologne, Germany;*
[5]*Department of Urology, Free University Berlin, D-12200 Berlin, Germany*

**Abstract** Two major transcripts of lymphoid enhancer factor-1 (LEF-1) have been described. The long isoform with β-catenin binding domain functions as a transcriptional enhancer factor. The short isoform derives from an intronic promoter and exhibits dominant negative activity. Recently, alterations of LEF-1 isoforms distribution have been described in colon cancer. In the current study we employed a quantitative real-time reverse transcription PCR method (TaqMan) to analyze expression of LEF-1 isoforms in a large cohort of human tumor (*n*=304) and tumor-free control samples (*n*=56). The highest expression level of LEF-1 was found in carcinoma samples whereas brain cancer samples expressed little. Expression of LEF-1 was different in distinct cancer types. For example, the mRNA level of LEF-1 was lower in testicular tumor samples compared with tumor-free control samples. Besides epithelial cancers, significant LEF-1 expression was also found in hematopoietic cells. In hematological malignancies, overall LEF-1 level was higher in lymphocytic leukemias compared with myeloid leukemias and normal hematopoiesis. However, acute myeloid leukemia and acute lymphocytic leukemia showed a significantly increased fraction of the oncogenic LEF-1 compared with chronic lymphocytic leukemia and chronic myeloid leukemia. Taken together, these data suggest that LEF-1 is abundantly expressed in human tumors and the ratio of the oncogenic and the dominant negative short isoform altered not only in carcinomas but also in leukemia.

**Key words** AML; β-catenin; isoform; lymphoid enhancer factor (LEF-1); solid tumor

Lymphoid enhancer factor-1 (LEF-1) is a member of the lymphoid enhancer factor/T-cell factor (LEF/TCF) family (LEF-1, TCF-1, TCF-3 and TCF-4) of high mobility group (HMG) transcription factors [1]. The human *LEF-1* gene spans at least 140 kb, and contains 12 exons and 11 introns with a large third intron (about 75 kb) that may contain an alternative exon. The *LEF-1* gene encodes at least two isoforms. Northern blotting analysis shows that the most abundant LEF-1 mRNA in cell lines is a 3.6 kb fragment with a 1.2 kb 5′ untranslated region (UTR), a 1.2 kb open reading frame and a 1.2 kb 3′ UTR, which encodes a 54 kDa nuclear protein with several functional domains, including a β-catenin binding domain, an HMG DNA binding domain and a transcription activation domain (CAD). In addition, the truncated LEF-1 isoform derives from an intronic promoter that drives expression of a dominant negative isoform, which lacks a β-catenin binding domain. This isoform was found in human thymus, testis and fetal brain tissues, but not in colon cancer and melanoma cells [2]. LEF-1 is a sequence-specific DNA-binding protein that has no transcriptional activation potential by itself [3]. It binds to a functionally important site in the T-cell receptor-alpha enhancer through an

Received: October 25, 2004 Accepted: January 13, 2005
This work is supported by grants from the José-Carreras Leukemia Foundation, the Deutsche Forschungsgemeinschaft (Mu 1328/2-3, Se 600/2-2, SFB293), the Deutsche Krebshilfe (10-1819-Mü2), the IZKF (Interdisziplinäres Zentrum für Klinische Forschung) at the University of Münster, and the Fund of Jiangsu University
*Corresponding authors:
Ping JI: Tel, +49-251-835-2994; Fax, +49-251-835-2673; E-mail, jiping6606@yahoo.com
Carsten MÜLLER-TIDOW: Tel, +49-251-835-2995; Fax, +49-251-835-2673; E-mail, muellerc@uni-muenster.de

HMG domain and confers maximal enhancer activity [4]. In addition, LEF/TCF proteins have been shown to interact with β-catenin as downstream mediators of the Wnt signal transduction pathway. In tumors, even in those cells from cancer tissues that are normally negative for LEF-1 expression, LEF-1 isoforms are frequently detected [3].

Recently, alterations in LEF-1 isoform distribution have been described in colon cancer [3]. The change in the fraction of the oncogenic compared with the dominant negative isoform is thought to increase the oncogenic activity. So far it is unknown whether this phenomenon is limited to colon cancer or can be observed in other cancers as well.

To define its role in human cancer, we analyzed the expression and fraction of the β-catenin-sensitive LEF-1 isoform in a large cohort of human cancer and normal organs. This study was conducted to investigate whether dysregulation of the β-catenin-sensitive LEF-1 isoform is a common feature of multiple cancer types.

## Material and Methods

### Patients and specimens

This study included tumor specimens and normal tissues from 360 patients with different types of cancer or controls, all of which were obtained during initial surgery or before chemotherapy. Samples were snap frozen in liquid nitrogen and stored at –80 °C. The number of specimens of each group was as follows: $CD34^+$ hematopoietic progenitor cells (*n*=5), normal bone marrow (*n*=7), lymphocytes (*n*=4), acute myeloid leukemia (AML) (*n*=82), acute lymphocytic leukemia (ALL) (*n*=21), chronic myeloid leukemia (CML) (*n*=11), chronic lymphocytic leukemia (CLL) (*n*=3), brain tumors (*n*=35), myeloma (*n*=7), breast cancer (*n*=12), cervical cancer (*n*=5), colon cancer (*n*=3), endometrial cancer (*n*=13), stomach cancer (*n*=12), ovarian cancer (*n*=8), esophageal cancer (*n*=5), non-small cell lung cancer (NSCLC) (*n*=63), lung controls (*n*=9), normal organs (*n*=23), Sertoli cell only syndrome (SCOS) (*n*=3), testicular cancer (*n*=24) and testis controls (*n*=5). In addition, the cDNAs of various normal organs (*n*=23) were commercially obtained (Clontech, Palo Alto, CA, USA).

### Total RNA isolation and cDNA preparation

The tumor samples were analyzed for the percentage of tumor cells by histology; only tumor biopsies with at least 70% cancer cells were used for subsequent analyses. Similarly, tumor-free control samples were confirmed by histological examination. For total RNA preparation, samples were disrupted into small pieces and RNA was isolated using TRIzol reagent (Life Technologies, Inc.). One microgram of RNA from each sample was reverse-transcribed using an oligo(dT) primer and MMLV reverse transcriptase according to the protocol of the manufacturer (Clontech, Palo Alto, CA, USA). The resulting cDNA was diluted to a total volume of 200 μl.

### Analyses of gene expression by real-time quantitative RT-PCR

The long LEF-1 isoform, both the long and short LEF-1 isoforms and an internal reference gene *GAPDH* were quantified according to a fluorescence-based real-time detection method (RT-PCR) using the HT7900 sequence detection system (TaqMan; Perkin-Elmer Applied Biosystems, Foster City, CA, USA). The PCR reaction mixture consisted of 600 nM each of the primers and 200 nM probe in a final volume of 22.5 μl. PCR conditions were at 50 °C for 10 s, 95 °C for 10 min, followed by 30 cycles at 95 °C for 15 s and 60 °C for 1 min.

The primer and probe sequences of LEF-1 isoforms were designed as follows. For the long LEF-1 isoform, primer 1 (from exon 1) 5′-AATCATCCCGGCCAGCA-3′ and primer 2 (from exon 2) 5′-TGTCGTGGTAGGGCTCCTC-3′ were used. Probe 1 is 6-FAM-5′-ACACGAGGTGGCCAGACAAGCACAA-3′-TAMRA. For both long and short LEF-1 isoforms, primer 5 (from exon 5) 5′-GTGTTGGACAGATCACCCCAC-3′ and primer 6 (from exon 6) 5′-CCTGAATCCACCCGGATC-3′ were used. Probe 2 is 6-FAM-5′-TCTTGGCTGGCAAGGTCAGCCTGTATATC-3′-TAMRA.

The primer and probe combinations were positioned to span an exon-exon junction [**Fig. 1(A)**]. When genomic DNA was used as a template, no bands were detected after PCR amplification. Initial template concentration could be calculated from the cycle number when the amount of PCR product passed a threshold set in the exponential phase of the PCR reaction. Relative gene expression levels were calculated using standard curves generated by serial dilutions of a mixture of cDNAs representing a wide variety of normal and tumor samples. The relative expression amounts of the long LEF-1 isoform and both LEF-1 isoforms were calculated by using the expression of *GAPDH* gene as an internal standard. The ratio of the long LEF-1 isoform was calculated by dividing the expression level of the long LEF-1 isoform by both LEF-1 isoforms. Two independent analyses were performed for each sample and for each gene.

### Data analysis

Statistical data analyses were performed using SPSS version 10.0. The Mann-Whitney *U*-test was used to compare differences of two groups and expression levels of several groups were compared with the Kruskal Wallis test. *P* value indicates two-sided comparison, and $P<0.05$ indicates statistically significant difference.

## Results

The *LEF-1* gene encodes two major isoforms with the longer one having oncogenic functions. The shorter one acts as a dominant negative mutant and is thought to function as a regulatory mechanism. In this study, we developed a quantitative RT-PCR assay to analyze LEF-1 isoform expression in a large collection of human cancers. One primer-probe combination was placed at the junction of exon 1 and exon 2, which resulted in the selective amplification of the β-catenin sensitive long isoform of LEF-1 (**Fig. 1**). Another primer-probe combination amplified a sequence spanning exons 5 and 6 and thus included both the β-catenin sensitive long isoform and the dominant negative short isoform of LEF-1. Expression levels of the two LEF-1 isoforms were determined by using standard curves of serial dilutions of a mixture of cDNAs representing a wide variety of normal and tumor samples. Expression levels of the LEF-1 isoforms were standardized to *GAPDH* expression level for the comparison of samples independent of the amount of total input cDNA. The ratio of LEF-1 isoform expression levels was subsequently calculated to indicate the relationship of the abundance of LEF-1 isoforms.

The PCR reactions were set up using a semi-automated pipetting system for pre-PCR processing and subsequent cycling in the HT7900 sequence detector. Each sample was analyzed for each gene of interest at least twice on independently prepared reaction plates. An excellent degree of correlation was obtained in the independent analyses. The correlation between the two analyses for the β-catenin dependent isoform was $r=0.95$ ($P<0.0001$) [**Fig. 2(A)**].

Initially, we compared expression of the long isoform with β-catenin binding domain and both the long and short LEF-1 isoforms in all 360 specimens, which we divided

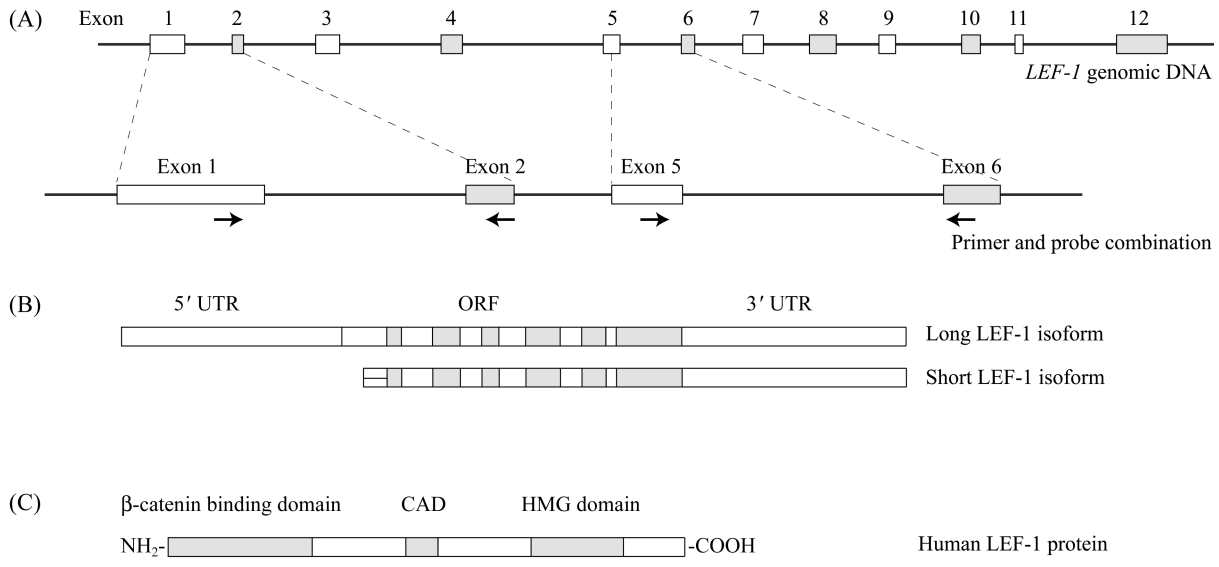


**Fig. 1 Diagram of human *LEF-1* genomic DNA and the mRNAs of its two major isoforms**

(A) Structure of *LEF-1* genomic DNA and position of the primers for TaqMan. The human *LEF-1* gene spans at least 140 kb, contains 12 exons and 11 introns, with the third 75 kb intron that may contain an alternative exon. The primer and probe combinations for RT-PCR were positioned to span the exon 1 and exon 2 junction, or to span the exon 5 and exon 6 junction. (B) Schematic diagram of the two major LEF-1 mRNA isoforms. The long LEF-1 isoform is a 3.6 kb fragment with a 1.2 kb 5′ UTR, a 1.2 kb open reading frame and a 1.2 kb 3′ UTR. The short LEF-1 isoform derives from an intronic promoter that drives expression of a dominant negative isoform. (C) The domain structure of human LEF-1 protein. The long LEF-1 isoform with β-catenin binding domain encodes a 54 kDa nuclear protein with several functional domains. The most highly conserved domain is the HMG DNA binding domain near the C-terminus. It is encoded by exon 8, exon 9 and exon 10. The next most highly conserved domain is a β-catenin binding domain at the extreme N-terminus.

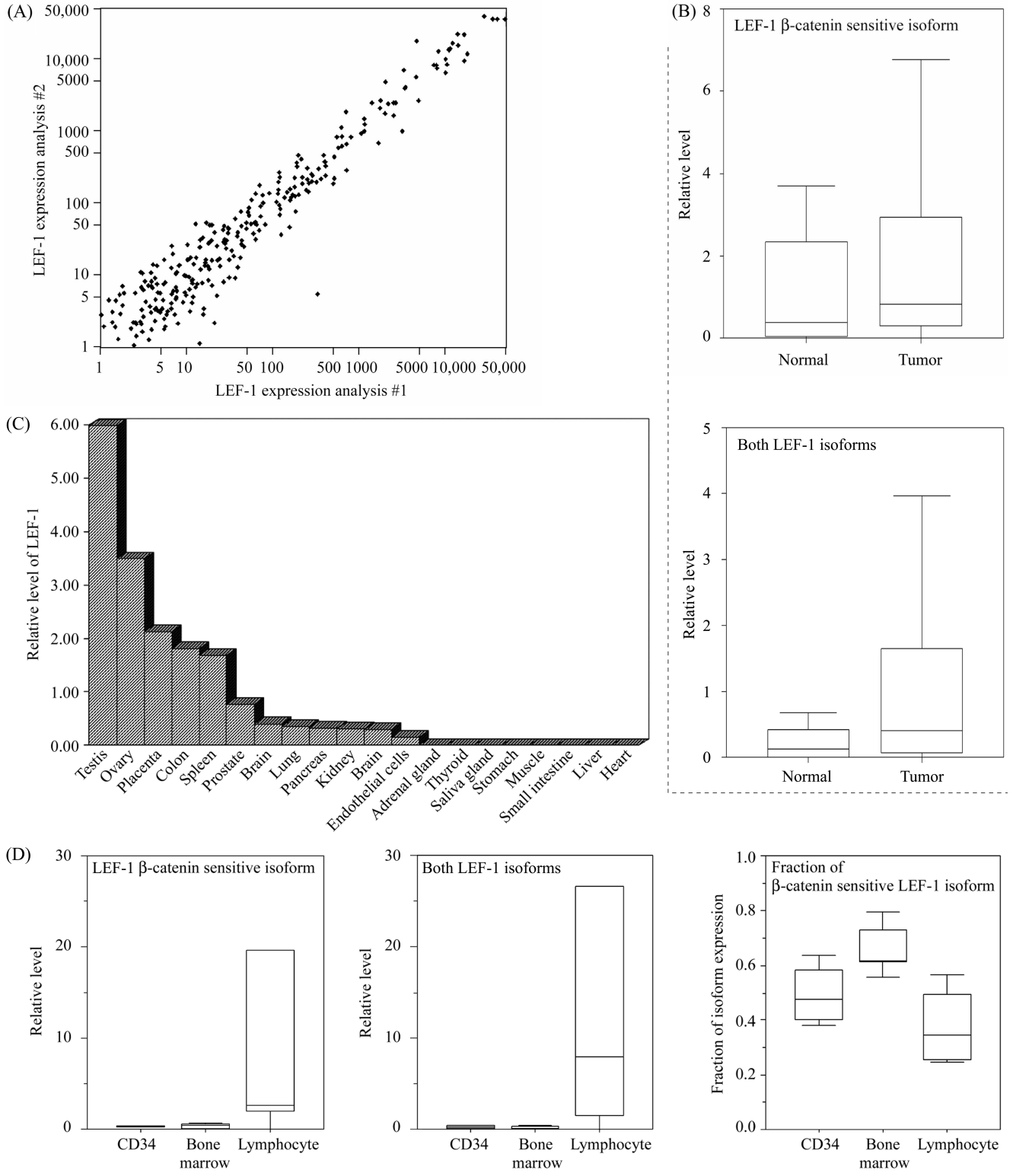


**Fig. 2 Analysis of LEF-1 expression in tumor tissues and normal control tissues**

(A) The real-time RT-PCR analyses yielded highly reproducible results. The results of two independent analyses of the long LEF-1 expression correlated closely ($r$=0.95, $P$<0.0001). (B) Analyses of all tumor ($n$=304) and control ($n$=56) samples for the long LEF-1 isoform and for both the long and short LEF-1 isoforms. Tumors expressed on average higher levels of the long LEF-1 isoform with β-catenin binding domain ($P$=0.015) and both LEF-1 isoforms ($P$=0.001). (C) Expression levels of the long LEF-1 isoform with β-catenin binding isoform in normal organs. (D) Expression levels of the long LEF-1 isoform and the fraction of the long LEF-1 isoform in hematopoietic cells. Lymphocytes expressed the highest levels of the long LEF-1 isoform and both LEF-1 isoforms.

into two groups: tumor samples ($n$=304) and tumor-free control samples ($n$=56). These analyses revealed that overall expression of the long LEF-1 isoform with β-catenin binding domain was higher in tumor samples compared with those in control samples ($P$=0.015) [**Fig. 2(B)**]. When we analyzed the long LEF-1 isoform expression in normal organs, the highest LEF-1 expression levels were found in testis, ovary and placenta [**Fig. 2(C)**]. No LEF-1 expression was detected in organs that do not show high proliferative activity in adult life (adrenal gland, thyroid, saliva gland, stomach, muscle, liver, and heart). Also, no LEF-1 was detected in small intestine, whereas activity was readily present in colon. In blood cells, high expression was noted in lymphocytes, but expression was also found in $CD34^+$ progenitor cells and normal bone marrow. The fraction of the long isoform versus both isoforms was lower in lymphocytes than in hematopoietic progenitors and bone marrow [**Fig. 2(D)**].

Further analyses of the cancer samples demonstrated that carcinomas expressed the highest LEF-1 levels [**Fig. 3(A)**].

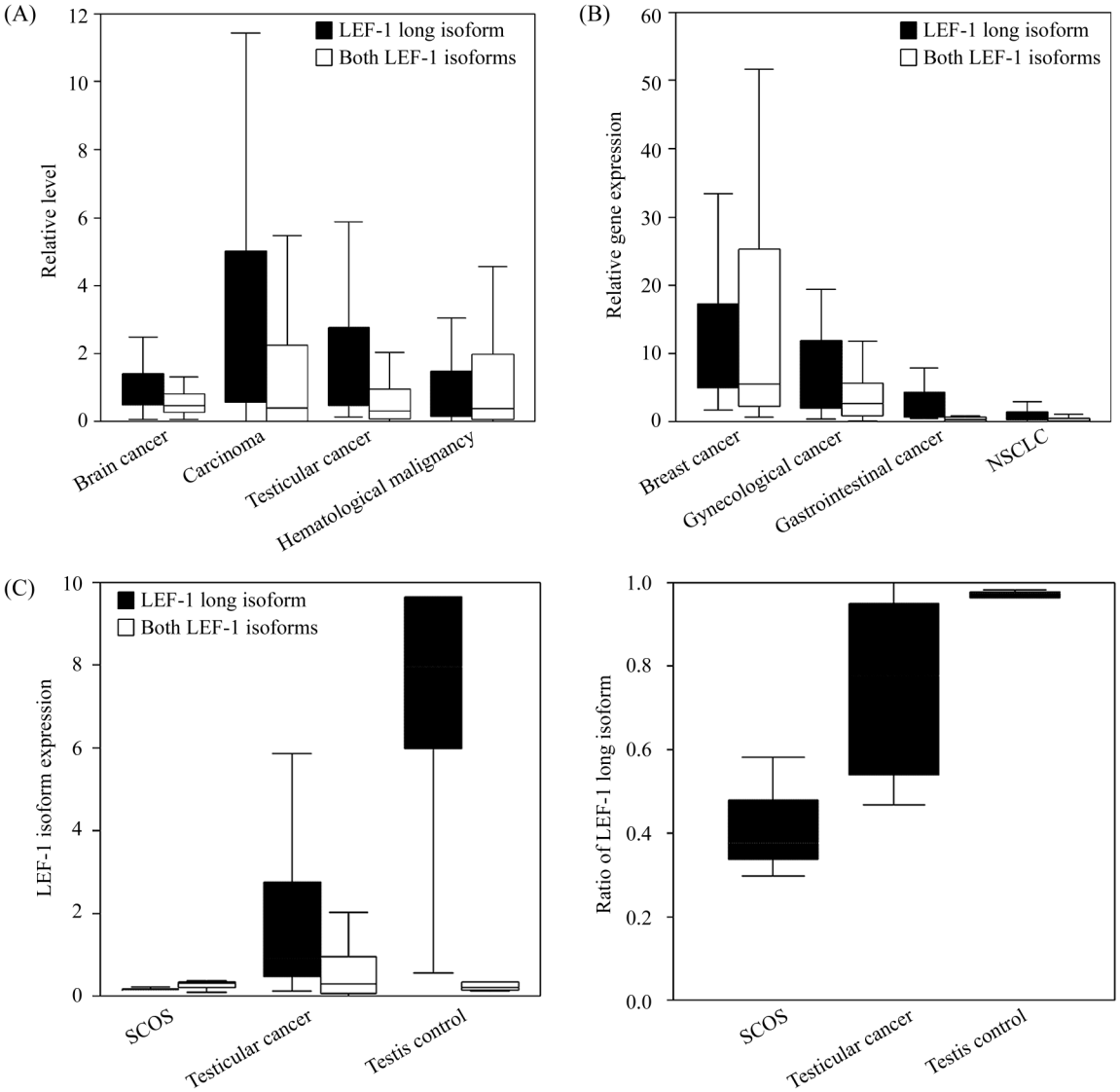


**Fig. 3 Expression of the long LEF-1 isoform with β-catenin binding domain in solid tumors**

(A) Expression of the long LEF-1 isoform with β-catenin binding domain in different tumor types. The highest LEF-1 levels were found in carcinomas. (B) Among the carcinomas, breast and gynecological tumors expressed the highest levels of LEF-1. (C) In testis tissue, the β-catenin-sensitive form was highly expressed during spermatogenesis. This is indicated by the lack of LEF-1 expression in samples lacking spermatogenesis (Sertoli cell only syndrome, SCOS). Interestingly, testicular cancer shows a significantly lower level of LEF-1 expression.

Among the carcinomas, breast and gynecological malignancies expressed the highest overall levels of LEF-1 isoforms [**Fig. 3(B)**]. In most solid tumors, LEF-1 expression level was either similar to expression levels found in the corresponding normal tissues or increased in tumors. One notable exception was testis tissue. High expression levels were found in normal testis tissue whereas expression dropped significantly in testicular tumors [**Fig. 3(C)**]. The analyses of samples lacking spermatogenesis (Sertoli cell only syndrome, SCOS samples) revealed that LEF-1 expression was found in testis tissue exclusively during spermatogenesis.

In hematological malignancies, the highest levels of LEF-1 were detected in CLL and ALL (**Fig. 4**). This corresponded well to the high LEF-1 levels found in normal lymphocytes. On the other hand, low LEF-1 levels were detectable in myeloma cells as well as in AML and CML. LEF-1 isoforms were predominantly expressed in CLL cells and were almost completely absent in AML (**Fig. 4**). As a result, the fraction of the long, oncogenic LEF-1 isoform was particularly high in the acute leukemias ALL and AML, whereas the ratio was very low in CML, myeloma and CLL (**Fig. 4**).

## Discussion

LEF/TCF signaling is a downstream event physiologically induced by Wnt-signaling pathway during development. In cancer, aberrant activation of this pathway occurs at multiple levels and plays an important role in pathogenesis of the disease [5,6].

In the current study, we developed a quantitative real-time RT-PCR assay to analyze changes in LEF-1 isoform expression in a wide range of human cancers. The main findings are as follows. First, human tumors, especially carcinomas, expressed higher levels of LEF-1 compared with normal tissue. Second, high levels of LEF-1 were found during spermatogenesis and LEF-1 expression was significantly reduced in testicular cancer. Third, in hematological malignancies an increased ratio of the oncogenic LEF-1 isoform compared with both isoforms was detected in acute leukemias [7,8]. These findings indicate that alterations of LEF-1 isoform distribution might be involved in aberrant Wnt pathway activation in acute leukemias.

Analyses of gene expression levels can be performed at the mRNA as well as on the protein level. The level of LEF-1 mRNA, measured by Northern blotting, has been studied previously in colon cancer [4]. LEF-1 mRNA was easily detected in the thymus by Northern blotting, but very little mRNA can be detected in any other tissues [1]. It is important to note that Northern blotting data can be misleading, and expression of LEF-1 has been detected by RT-PCR or *in situ* hybridization of tissues that appeared negative by Northern blotting analysis [9]. For clinical

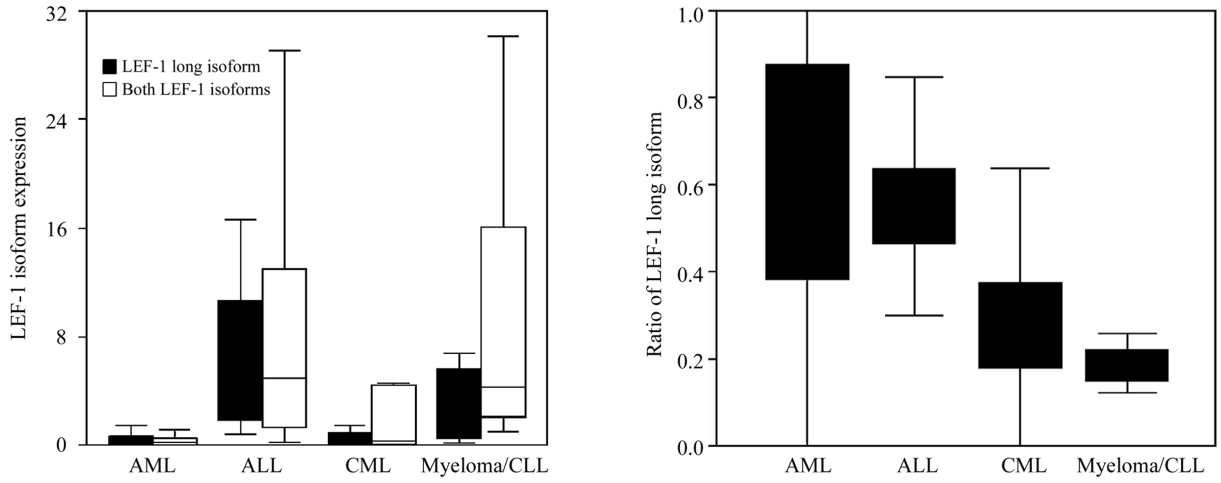


**Fig. 4 LEF-1 expression in hematological malignancies**

The long LEF-1 isoform with β-catenin binding domain was expressed in all leukemias and in multiple myeloma, but the highest levels were found in chronic lymphocytic leukemia (CLL) and in acute lymphocytic leukemia (ALL). High levels of both LEF-1 isoforms were also present in CLL and ALL samples. Analyses of the fraction of the long LEF-1 isoform revealed, that the relative amount of the oncogenic isoform was much higher in the acute leukemias compared to chronic leukemias and multiple myelomas.

specimens, analyses by RT-PCR provide a rapid way of detecting the presence or absence of specific transcripts. However, before the advent of real-time RT-PCR, the accurate analysis of the amount of transcripts in the specimen was time-consuming and error prone. In our study, we have used the 5′ nuclease assay to determine quantitatively the level of expression of the long LEF-1 isoform with β-catenin binding domain in a large cohort of human cancer ($n$=360) and normal controls ($n$=54). Despite the convenience and accuracy of real-time RT-RT-PCR, several factors have to be taken into account to avoid potential pitfalls that might hamper the quality of the data. To minimize the problem of normal cell contamination of the tumor samples, we only used specimens that contained a high percentage (>70%) of tumor cells. To exclude analysis of genomic DNA that might contaminate the RNA preparation, the probes were designed to cover an exon-exon junction of the *LEF-1* gene. In addition, all samples were quantitated according to a standard curve, which was run on every PCR plate. The same standard samples were used for each PCR plate to standardize results among different plates. All samples were analyzed twice on independently prepared PCR reaction plates. This method has been shown to be an easy, fast and reliable alternative to the more traditional methods for the determination of various gene expressions [10].

Wnt proteins represent a family of secreted signal molecules that are expressed in diverse tissues and have been shown to influence multiple processes in vertebrate and invertebrate development [11]. Dysregulation of components and constitutive activation of the Wnt signaling pathway can have potent oncogenic effects in various tissues, such as colon, breast, and NSCLC [12–14]. β-catenin is thought to be the key mediator of the Wnt signaling pathway [15]. The level of β-catenin in the cell is regulated by its association with the tumor suppressor molecule adenomatous polyposis coli (APC) [16], axin [17] and glycogen synthase kinase 3β (GSK-3β) [18]. Phosphorylation of β-catenin by the APC-axin-GSK-3β complex leads to its degradation by the ubiquitin-proteasome system [19, 20]. LEF-1 blocks APC-mediated β-catenin nuclear export and activates transcription of various transforming genes, including cyclin D1, Myc, MMP7, TCF7 and LEF-1 itself [21–24]. LEF-1 has been established as an important factor in Wnt signaling and tumorigenesis [4]. LEF-1 and other TCF family members are implicated in the development of cancer by their association with β-catenin.

The role of Wnt-signaling in hematopoiesis and leukemia pathogenesis is not well understood. Wnt proteins were able to determine the cell fate of hematopoietic progenitors and expression of several members of the Wnt family led to an expansion of multilineage progenitor cells [24]. Several recent findings provided hints that hematopoiesis and leukemia might be more closely associated with the Wnt-signaling pathway than previously anticipated. For example, Wnt signaling prevents adipocyte differentiation by inhibiting C/EBPα. C/EBPα is a major inducer of granulopoiesis and is frequently mutated in the FAB M2 subtype of AML [25]. Also, vitamin D3 that induces differentiation of U937 leukemia cells promotes colon carcinoma cell differentiation by inducing E-cadherin and by inhibiting β-catenin signaling [26]. Finally, all-*trans* retinoic acid (ATRA) was shown to inhibit β-catenin-TCF/LEF signaling [27]. In microarray analyses we have found that γ-catenin as well as several TCF factors are induced by AML associated fusion proteins [28]. Thus our finding that acute leukemias showed an increased fraction of the oncogenic LEF-1 isoform fits well into the emerging picture of TCF/LEF signaling in leukemogenesis.

## References


1 Nawshad A, Hay ED. TGF$\beta_3$ signaling activates transcription of the LEF1 gene to induce epithelial mesenchymal transformation during mouse palate development. J Cell Biol, 2003, 163(6): 1291–1301

2 Oosterwegel MA, van de Wetering ML, Holstege FC, Prosser HM, Owen MJ, Clevers HC. TCF-1, a T cell-specific transcription factor of the HMG box family, interacts with sequence motifs in the TCR beta and TCR delta enhancers. Int Immunol, 1991, 3(11): 1189–1192

3 Hovanes K, Li TWH, Waterman ML. The human LEF-1 gene contains a promoter preferentially active in lymphocytes and encodes multiple isoforms derived from alternative splicing. Nucleic Acids Res, 2000, 28(9): 1994–2003

4 Hovanes K, Li TWH, Munguia JE, Truong T, Milovanovic T, Lawrence Marsh J, Holcombe RF *et al*. β-catenin-sensitive isoforms of lymphoid enhancer factor-1 are selectively expressed in colon cancer. Nat Genet, 2001, 28(1): 53–57

5 Kobielak A, Kobielak K, Trzeciak WH. A novel isoform of human lymphoid enhancer-binding factor-1 (LEF-1) gene transcript encodes a protein devoid of HMG domain and nuclear localization signal. Acta Biochim Pol, 2001, 48 (1): 221–226

6 Giese K, Kingsley C, Kirshner JR, Grosschedl R. Assembly and function of a TCR alpha enhancer complex is dependent on LEF-1-induced DNA bending and multiple protein-protein interactions. Genes Dev, 1995, 9(8): 995–1008

7 Bruhn L, Munnerlyn A, Grosschedl R. ALY, a context-dependent coactivator of LEF-1 and AML-1, is required for TCR alpha enhancer function. Genes Dev, 1997, 11(5): 640–653

8 Love JJ, Li X, Case DA, Giese K, Grosschedl R, Wright PE. Structural basis for DNA bending by the architectural transcription factor LEF-1. Nature, 1995, 376(6543): 791–795

9 Kratochwil K, Dull M, Farinas I, Galceran J, Grosschedl R. LEF1 expression is activated by BMP-4 and regulates inductive tissue interactions in tooth and hair development. Genes Dev, 1996, 10(11): 1382–1394

10 Muller-Tidow C, Metzger R, Kugler K, Diederichs S, Idos G, Thomas M, Dockhorn-Dworniczak B *et al*. Cyclin E is the only cyclin-dependent kinase 2-associated cyclin that predicts metastasis and survival in early stage non-small cell lung cancer. Cancer Res, 2001, 61(2): 647–653
11 Huelsken J, Birchmeier W. New aspects of Wnt signaling pathways in higher vertebrates. Curr Opin Genet Dev, 2001, 11(5): 547–553
12 Kim K, Lu Z, Hay ED. Direct evidence for a role of beta-catenin/LEF-1 signaling pathway in induction of EMT. Cell Biol Int, 2002, 26(5): 463–476
13 Behrens J. Control of β-catenin signaling in tumor development. Ann NY Acad Sci, 2000, 910: 21–33; discussion 33–35
14 Webster MT, Rozycka M, Sara E, Davis E, Smalley M, Young N, Dale TC *et al*. Sequence variants of the axin gene in breast, colon, and other cancers: An analysis of mutations that interfere with GSK3 binding. Genes Chromosomes Cancer, 2000, 28(4): 443–453
15 Lipinski KS, Djeha HA, Gawn J, Cliffe S, Maitland NJ, Palmer DH, Mountain A *et al*. Optimization of a synthetic β-catenin-dependent promoter for tumor-specific cancer gene therapy. Mol Ther, 2004, 10(1): 150–161
16 Henderson BR, Galea M, Schuechner S, Leung L. Lymphoid enhancer factor-1 blocks adenomatous polyposis coli-mediated nuclear export and degradation of β-catenin. Regulation by histone deacetylase 1. J Biol Chem, 2002, 277(27): 24258–24264
17 Hart MJ, de los Santos R, Albert IN, Rubinfeld B, Polakis P. Downregulation of beta-catenin by human Axin and its association with the APC tumor suppressor, beta-catenin and GSK3 beta. Curr Biol, 1998, 8(10): 573–581
18 Karim R, Tse G, Putti T, Scolyer R, Lee S. The significance of the Wnt pathway in the pathology of human cancers. Pathology, 2004, 36(2): 120–128
19 van Noort M, Meeldijk J, van der Zee R, Destree O, Clevers H. Wnt signaling controls the phosphorylation status of beta-catenin. J Biol Chem, 2002, 277(20): 17901–17905
20 Aberle H, Bauer A, Stappert J, Kispert A, Kemler R. beta-catenin is a target for the ubiquitin-proteasome pathway. EMBO J, 1997, 16(13): 3797–3804
21 He TC, Sparks AB, Rago C, Hermeking H, Zawel L, da Costa LT, Morin PJ *et al*. Identification of c-MYC as a target of the APC pathway. Science, 1998, 281(5382): 1509–1512
22 Tetsu O, McCormick F. Beta-catenin regulates expression of cyclin D1 in colon carcinoma cells. Nature, 1999, 398: 422–426
23 Crawford HC, Fingleton BM, Rudolph-Owen LA, Goss KJ, Rubinfeld B, Polakis P, Matrisian LM. The metalloproteinase matrilysin is a target of beta-catenin transactivation in intestinal tumors. Oncogene, 1999, 18: 2883–2891
24 Brabletz T, Jung A, Dag S, Hlubek F, Kirchner T. Beta-catenin regulates the expression of the matrix metalloproteinase-7 in human colorectal cancer. Am J Pathol, 1999, 155: 1033–1038
25 Pabst T, Mueller BU, Harakawa N, Schoch C, Haferlach T, Behre G, Hiddemann W *et al*. AML1-ETO downregulates the granulocytic differentiation factor C/EBPalpha in t(8;21) myeloid leukemia. Nat Med, 2001, 7(4): 444–451
26 Palmer HG, Gonzalez-Sancho JM, Espada J, Berciano MT, Puig I, Baulida J, Quintanilla M *et al*. Vitamin D(3) promotes the differentiation of colon carcinoma cells by the induction of E-cadherin and the inhibition of beta-catenin signaling. J Cell Biol, 2001, 154(2): 369–387
27 Easwaran V, Pishvaian M, Salimuddin, Byers S. Cross-regulation of beta-catenin-LEF/TCF and retinoid signaling pathways. Curr Biol, 1999, 9(23): 1415–1418
28 Muller-Tidow C, Steffen B, Cauvet T, Tickenbrock L, Ji P, Diederichs S, Sargin B *et al*. Translocation products in acute myeloid leukemia activate the Wnt signaling pathway in hematopoietic cells. Mol Cell Biol, 2004, 24(7): 2890–2904

Edited by
**Ding-Gan LIU**