Regular Article

LYMPHOID NEOPLASIA

Single nucleotide variation in the *TP53* 3′ untranslated region in diffuse large B-cell lymphoma treated with rituximab-CHOP: a report from the International DLBCL Rituximab-CHOP Consortium Program

Yong Li,[1,2] Michael W. Gordon,[1] Zijun Y. Xu-Monette,[2] Carlo Visco,[3] Alexander Tzankov,[4] Dehui Zou,[5] Lugui Qiu,[5] Santiago Montes-Moreno,[6] Karen Dybkaer,[7] Attilio Orazi,[8] Youli Zu,[9] Govind Bhagat,[10] Kristy L. Richards,[11] Eric D. Hsi,[12] William W. L. Choi,[13] J. Han van Krieken,[14] Qin Huang,[15] Weiyun Ai,[16] Maurilio Ponzoni,[17] Andrés J. M. Ferreri,[17] Jane N. Winter,[18] Ronald S. Go,[19] Miguel A. Piris,[6] Michael B. Møller,[20] Lin Wu,[21] Michael Wang,[22] Kenneth S. Ramos,[1] L. Jeffrey Medeiros,[2] and Ken H. Young[2]

[1]Department of Biochemistry and Molecular Biology, University of Louisville School of Medicine, Brown Cancer Center, Louisville, KY; [2]Department of Hematopathology, The University of Texas MD Anderson Cancer Center, Houston, TX; [3]Department of Hematology, San Bortolo Hospital, Vicenza, Italy; [4]Department of Pathology, University Hospital, Basel, Switzerland; [5]Institute of Hematology, Chinese Academy of Medical Science and Peking Union Medical College, Tianjin, China; [6]Department of Pathology, Hospital Universitario Marqués de Valdecilla/IFIMAV, Santander, Spain; [7]Department of Hematology, Aarhus University Hospital, Aalborg, Denmark; [8]Department of Pathology, Weill Medical College of Cornell University, New York, NY; [9]Department of Pathology, The Methodist Hospital, Houston, TX; [10]Department of Pathology, Columbia University School of Medicine, New York, NY; [11]Department of Hematology, University of North Carolina School of Medicine, Chapel Hill, NC; [12]Department of Pathology, Cleveland Clinic, Cleveland, OH; [13]Department of Pathology, University of Hong Kong Li Ka Shing Faculty of Medicine, Hong Kong, China; [14]Department of Pathology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; [15]Department of Pathology, City of Hope National Medical Center, Duarte, CA; [16]Department of Hematology, University of California San Francisco School of Medicine, San Francisco, CA; [17]Departments of Hematology and Pathology, San Raffaele H. Scientific Institute, Milan, Italy; [18]Department of Hematology, Feinberg School of Medicine, Northwestern University, Chicago, IL; [19]Department of Hematology, Gundersen Lutheran Health System, La Crosse, WI; [20]Department of Pathology, Odense University Hospital, Odense, Denmark; [21]Department of Genomic Oncology, Roche Molecular Systems Inc., Pleasanton, CA; and [22]Department of Lymphoma and Myeloma, The University of Texas MD Anderson Cancer Center, Houston, TX

Key Points

- *TP53* 3′UTR variations demonstrate prognostic value in DLBCL.

We identified multiple single nucleotide variants (SNVs) in the *TP53* 3′ untranslated region (3′UTR) in tumor specimens from 244 patients with diffuse large B-cell lymphoma (DLBCL). Patients carrying a wild-type *TP53* coding sequence (CDS) and 1 or more 3′UTR SNVs had a better 5-year survival rate than patients carrying a wild-type CDS and the reference 3′UTR, yet there is no statistically significance difference in overall survival (OS). In contrast, 3′UTR variation predicted poorer OS for patients with a mutant *TP53* CDS. We then sequenced *TP53* 3′UTR in 247 additional DLBCL patients as a validation set. Altogether, we identified 187 novel SNVs; 36 occurred at least twice. Most of the newly identified 3′UTR SNVs were located at sites that are complementary to seed sequences of microRNAs (miRNAs) that are predicted or experimentally known to target *TP53*. Three SNVs disrupt the seed match between miR-125b and the *TP53* 3′UTR, thereby impeding suppression of p53 by this miRNA. In addition, a germline SNV (rs78378222) located in the *TP53* polyadenylation signal resulted in downregulation of both p53 messenger RNA and protein levels and reduction of cellular apoptosis. This study is the first to demonstrate the prognostic value of the *TP53* 3′UTR in cancer. (*Blood*. 2013;121(22):4529-4540)

Introduction

The tumor suppressor p53, encoded by *TP53*, is recognized as the guardian of the human genome. p53 responds to diverse stressors by regulating many downstream target genes that promote cellular DNA repair, apoptosis, cell cycle arrest, and senescence. Restoring p53 function alone is sufficient to cause regression of several types of cancer in mice.[1,2] This characteristic suggests that p53 itself or the p53 pathway must be derailed for tumor development, genetically or otherwise. Three distinct sets of nucleotide alterations can occur in the human *TP53* gene: tumor-associated (somatic) mutations, germline Li-Fraumeni mutations, and germline *p53* polymorphisms.[3,4] Both tumor-associated mutations and Li-Fraumeni mutations occur in the

p53 coding sequence (CDS) and produce mutant p53 proteins that lack most or all of the normal p53 functions and confer oncogenic properties.[5] For instance, a missense base substitution at codon 273 of human *TP53* (R273H) is frequently observed in somatic tumors and in tumors from Li-Fraumeni syndrome patients. Mouse models demonstrate that this mutant *p53* allele has enhanced oncogenic potential that cannot be explained by simple loss of p53 function.[6,7]

In general, p53 CDS mutations contribute to oncogenesis by 2 distinct mechanisms: (1) mutant alleles can have dominant negative effects on wild-type (WT) p53 function; and (2) mutant p53 protein can actively promote tumorigenesis independently of WT p53

function. It is accepted that the WT *TP53* CDS produces native p53 tumor suppressor, whereas most *TP53* CDS mutations produce oncogenic mutant p53. By contrast, only a few of the >200 naturally occurring germline variants of *TP53* in human populations cause measurable perturbation of p53 function.[3] More than 90% of these p53 variants occur in 10 introns alleged to have no cancer-related biological consequences. None of the 20 variants in the *TP53* CDS have been linked reproducibly to cancer predisposition.[3,4] The best-studied *TP53* CDS polymorphism is codon 72 (R72P), owing to an earlier finding that human papillomavirus causes more efficient degradation of p53^{R72} than p53^{P72}.[8] However, few associations between this polymorphism and various types of cancer have been found.[3] Sequence variation in the untranslated regions (UTRs) of the *TP53* gene has not been extensively studied.

Diffuse large B-cell lymphoma (DLBCL) is the most common type of lymphoma and is clinically heterogeneous with subsets of patients with better or worse prognosis.[9,10] Biologically, DLBCL has been known to be heterogeneous with germinal center B-cell (GCB) and activated B-cell (ABC) subtypes. Recent advancements in next-generation sequencing have further broadened our understanding of genetic lesions in DLBCL. Three DLBCL-sequencing projects have provided a wealth of information on whole genomes, exomes, and/or transcriptomes for more than 100 DLBCL patient specimens, some with matched healthy tissues as controls.[11-13] These studies have identified mutations in more than 100 genes not implicated previously in DLBCL pathogenesis, including genes involved in chromatin modification and immune regulation. The *TP53* gene (the CDS) was found to be mutated in ~20% of DLBCL cases in all 3 deep-sequencing projects. However, *MLL2*,[11,13] *CREBBP*,[11] *EZH2*,[12] *BCL2*,[12] *GNA13*,[13] *BTG1*,[13] *PIM1*,[13] and *PCLO*[13] were found to be mutated more frequently than the *TP53* CDS in some of these studies. These findings challenge the notion that *TP53* is the most commonly mutated gene in cancer[14,15] at least within the context of DLBCL. In addition, noncoding genomic sequences have been largely overlooked in these sequencing projects.[11,12]

In a previous study, we found that ~20% of DLBCL patients who received cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) chemotherapy had *TP53* CDS mutations and that these mutations adversely affected prognosis.[16] To provide an in-depth analysis of DLBCL patients treated with CHOP plus the monoclonal antibody rituximab (*R*-CHOP), we assembled a multicenter study designated as the DLBCL *R*-CHOP Collaborative Consortium. We reported that *TP53* CDS mutations predicted poorer survival in 506 (largest-ever cohort) *R*-CHOP–treated DLBCL patients with either the GCB or the ABC subtype, and that *TP53* monoallelic deletion and loss of heterozygosity did not confer worse survival.[17] These findings suggested that assessment of *TP53* CDS mutation status can be useful for stratifying patients receiving *R*-CHOP treatment into distinct prognostic subsets.

In this study, we retrospectively sequenced the *TP53* 5′UTR and 3′UTR in DLBCL patients to determine if *TP53* 3′UTR sequence alterations occur in cancer patients and to assess their potential prognostic importance. We also evaluated the potential impact of *TP53* 3′UTR sequence variation in combination with CDS mutations, and we determined the functional significance of 3′ UTR variants in p53 regulation.

Materials and methods

Patients

For the initial study group, we randomly selected 244 patients (training set) with DLBCL from a total of 506 de novo DLBCL patients treated with *R*-CHOP, whose *TP53* CDSs had been sequenced in a previous study by our consortium.[17]

For the validation set, tumor specimens were also obtained from 247 DLBCL patients receiving *R*-CHOP treatment in the consortium program, none of whom were included in the 506 DLBCL patients in the previous p53 CDS study.[17]

All cases were reviewed by a group of hematopathologists (S.M.M., M.A.P., M.B.M., A.T., and K.H.Y.), and the diagnoses confirmed by World Health Organization classification criteria. The current study and material transfer agreement were approved by the institutional review board of each participating center. The overall collaboration was approved by the Institutional Review Board of The University of Texas MD Anderson Cancer Center, Houston, Texas. This study was conducted in accordance with the Declaration of Helsinki.

All of these patients have undergone molecular and phenotypic classification with available clinical data. There are no statistical differences in regard to clinical parameters (age, gender, stage, lactic dehydrogenase level, tumor size, performance status, chemotherapy regimen, survival time, and recurrence rate), GCB/ABC classification and distribution, and survival among 3 groups (244 cases in the training set, 247 cases in the validation set, and unselected remaining 262 cases).

DNA sequencing and cell biology

Genomic DNA was extracted from formalin-fixed, paraffin-embedded tissue samples using the HighPure paraffin DNA extraction kit (Qiagen). The *TP53* UTRs were sequenced bidirectionally using the Sanger method at Polymorphic DNA Inc. (Alameda, CA). All sequences were compared with the *TP53* reference sequence (NM_000546.5). Single nucleotide variants (SNVs) in the 3′UTR were grouped into single nucleotide polymorphisms (SNPs) and novel SNVs (nSNVs). Any nucleotide change listed in the dbSNP database was considered an SNP; the remainder (none occurring in ≥1% of patients) were considered nSNVs.

The p53-null human lung adenocarcinoma cancer cell line H1299 was obtained from the American Type Culture Collection (Manassas, VA). For mutational analysis, the p53 expression vector pCMV-p53 (Clontech, Mountain View, CA) was modified to contain *TP53* 5′ and 3′UTRs as well as the 2.5-kb genomic DNA downstream of the 3′UTR. The resulting constructs were designated as TP53Ext, TP53ExtC^{1170}T, and TP53ExtA^{1175}C, respectively. MicroRNA (miRNA) expression vectors and Western blotting analysis were described previously[18] and are included in the supplemental Materials and methods (see the *Blood* Web site).

Statistical analysis

All statistical calculations were performed using GraphPad Prism 5. Overall survival (OS) was calculated from time of diagnosis to time of death from any cause or last follow-up. Progression-free survival (PFS) was calculated from time of diagnosis to time of progression or death from any cause. The actuarial probabilities of OS and PFS[17] were determined using the Kaplan-Meier method, and differences were compared using the log-rank test. The 5-year survival and 5-year PFS rates describe the percentage of patients that are alive 5 years after their cancer is diagnosed; they were compared using Fisher's exact test. All differences with $P \leq .05$ were considered statistically significant.

Results

Distribution and frequency of SNVs in *TP53* UTRs in 244 de novo DLBCL patients

In the initial group of 244 DLBCL samples, we identified 128 SNVs in the UTRs of 107 patients, with 14 in the 5′UTR and 114 in the 3′UTR (Figure 1A; supplemental Table 1). Six 3′UTR SNVs have been reported as SNPs in dbSNP and occurred 37 times (Table 1). We designated the newly found noncoding SNVs in the *TP53* gene as nSNVs. A total of 108 nSNVs were identified with 121 occurrences
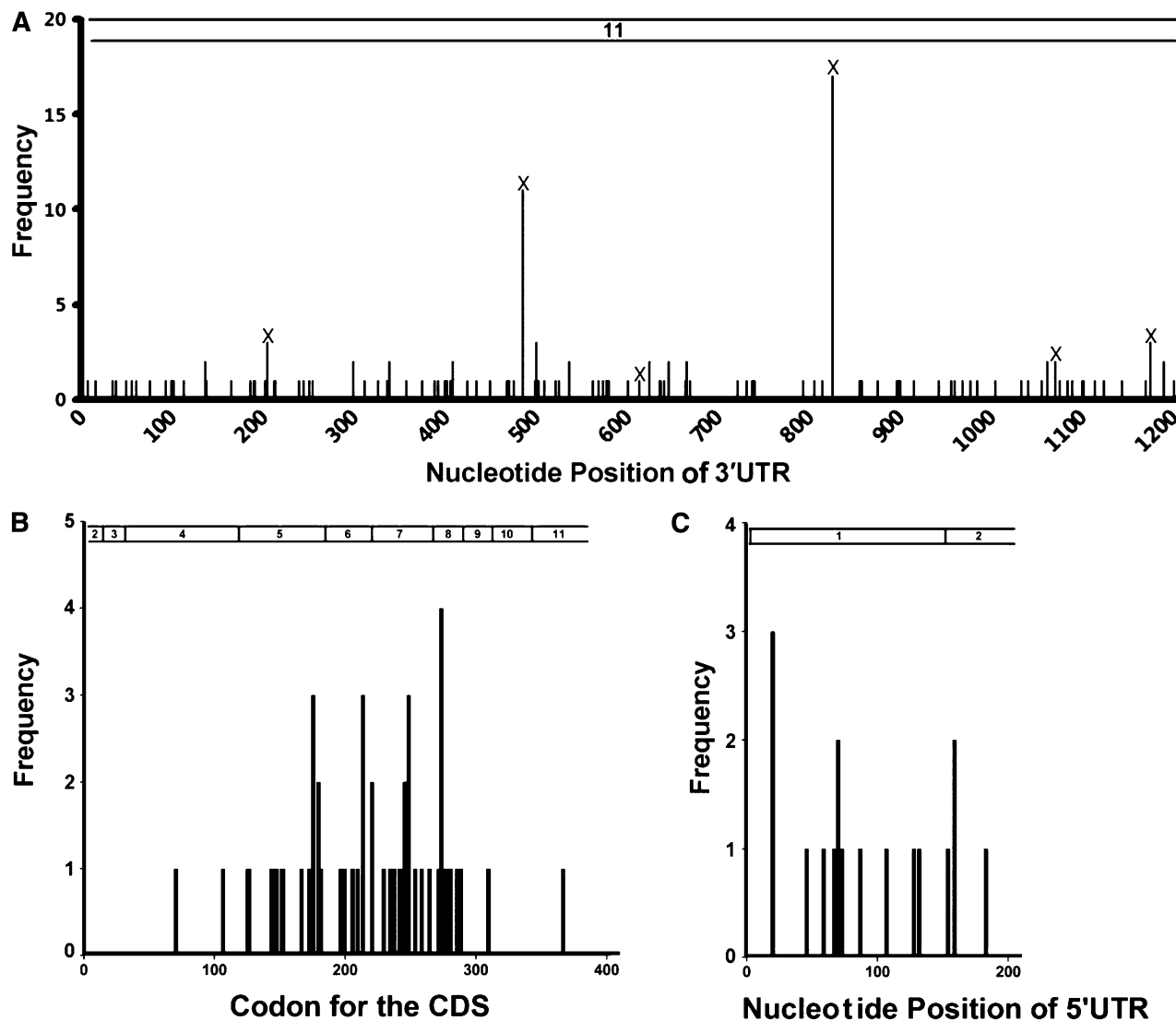
Figure 1. Distribution of variation in the *TP53* gene in 244 de novo DLBCL patients. (A) Distribution of SNVs in the 3′UTR (part of exon 11; nucleotides 1385-2591 of NM_000546.5 of the TP53 reference mRNA). (B) Distribution of CDS mutations in exons 3 to 10 and part of exons 2 and 11 (top bar; nucleotides 203-1384 of NM_000546.5). (C) Distribution of 5′UTR SNVs in exon 1 and part of exon 2, nucleotides 1-202 of NM_000546.5. Numbers on the top indicate exons.

in the 3′UTR in 76 patients. More patients had at least 1 nSNV in the 3′UTR (n = 76, 31%) than they had nonsynonymous mutations in the CDS (n = 49, 20%). In the 76 patients with 3′UTR nSNVs, 32 (42%) had multiple lesions, compared with 2 of 49 (4%) patients with 2 or more CDS mutations. Ten of the *TP53* 3′UTR nSNVs occurred twice, and 1 occurred 3 times (Figure 1A). The 5′UTR SNVs occurred at a much lower frequency (n = 15 patients, 6%; supplemental Table 1; none of them reported in dbSNP) and were not further analyzed in the current study.

Most *TP53* CDS somatic mutations are clustered within exons 5 to 8.[19] Although the nucleotide length (544 bp) of exons 5 to 8 accounts for only ~46% of the coding exons (1182 bp), mutations in these exons account for 94% of all CDS somatic mutations.[19] Our analysis of *TP53* CDS in the 506 DLBCL patients confirmed the uneven codon distribution of single nucleotide missense substitutions: mutations in exons 5 to 8 account for 90% of all mutations in coding exons (exons 3-10 plus parts of exon 2 and exon 11).[17] In the initial study group of 244 patients, 92% of all CDS mutations were found in exons 5 to 8 (45 of 49 cases, Figure 1B). In contrast, 3′UTR nSNVs were evenly dispersed across the

1207-bp 3′UTR in the 76 patients with nSNVs (Figure 1A). The distribution of SNVs in the 5′UTR in the 244 cases was similar to that in the 3′UTR (Figure 1C). These data suggest that UTR variants in the *TP53* gene are distributed more evenly than are CDS mutations.

Survival in relation to clinical characteristics and *TP53* 3′UTR variation

The clinical and biological characteristics of the 244 DLBCL patients and their impact on OS and PFS are summarized in supplemental Table 2. We first verified that no adverse clinical variables were associated with variation in the 3′UTR. When patients were divided based on the presence of *TP53* 3′UTR lesions (SNPs only, nSNVs only, or any SNVs [either SNP or nSNV]), no significant difference in OS or PFS was found (Figure 2). *TP53* CDS mutations (MUT-CDS) predicted poorer OS (P = .0028) and PFS (P = .0086) in this 244-patient cohort (Figure 3A-B). We then divided patients into 2 groups according to the p53 CDS status and analyzed survival in relation to SNVs. We found that SNPs in the *TP53* 3′UTR had no impact on

Table 1. Frequency of known *TP53* 3′UTR SNPs in DLBCL cohorts

SNP ID	Position†	MAF‡	Heterozygous frequency	Validation§	SNP occurrence* First group (244)	Second group (247)
rs8065799	9 A>C	N/A	N/A	—	0	0
rs1042526	37 A>T	N/A	N/A	Precious	0	0
rs35919705	105 C>T	0.010	0.015	Frequency	0	0
rs16956880	205 G>A	0.032	0.024	Cluster, Frequency, 1000Genome	3	2
rs79516338	309 G>A	N/A	0.500	Frequency	0	0
rs34486624	314 G>A	N/A	0.020	Frequency	0	0
rs17881366	328 G>A	0.026	0.010	Cluster, Frequency, 1000Genome	0	0
rs4968187	485 G>A	0.035	N/A	Cluster, Frequency, 1000Genome Precious	11	5
rs916131	491 G>A	N/A	N/A	Precious	0	0
rs916132	514 T>C	N/A	N/A	Precious	0	0
rs1811095	534 C>T	N/A	N/A	Cluster	0	0
rs35944977	534-535 TC>CT	N/A	N/A	—	0	0
rs1811096	535 C>T	N/A	N/A	—	0	1
rs17886358	569-570 GT>del	N/A	0.435	Cluster, Frequency	0	0
rs34607064	595 C>del	N/A	N/A	—	0	0
rs66759322	611 A>del	N/A	N/A	—	0	0
rs17879353	613 C>A	0.007	N/A	Cluster, Frequency	1	1
rs1042535	634 C>CG>G>GA	N/A	N/A	—	0	0
rs1794291	635 A>G	N/A	N/A	Cluster	0	0
rs34182553	674 C>del	N/A	N/A	—	0	0
rs77011241	773 C>T	N/A	0.389	Frequency, 1000Genome	0	0
rs35940853	798 T>del	N/A	N/A	—	0	0
rs17884306	826 G>A	0.044	N/A	Cluster, Frequency, 1000Genome, Precious	17	24
rs17884586	855 C>T	N/A	0.012	—	0	0
rs35659787	886 G>A	N/A	0.10	Frequency	0	0
rs55817367	925 C>T	0.007	N/A	1000Genome	0	0
rs1794292	995 G>C	N/A	N/A	—	0	0
rs1794293	1057 C>G	N/A	N/A	—	0	0
rs114831472	1070 C>T	0.032	0.065	1000Genome	2	1
rs78378222	1175 A>C	0.010	N/A	Cluster, Frequency, 1000Genome	3	4

*Minor allele found in the initiation set (244 cases) or the validation set (247 cases). Some patients possess more than 1 SNP. Underlining in column 2 indicates SNPs found in the cohort.

†Counted from first nucleotide of the *TP53* 3′UTR; "del," deletion.

‡MAF, minor allele frequency; N/A, not available.

§Validation: "Cluster," multiple submissions mapped to the same location and clustered into 1 refSNP cluster and assigned a reference SNP ID number; "Frequency," validated by both population frequency data and genotype data; "1000Genome," validated by the 1000 Genomes Project; "Precious," cited as clinically relevant in the literature and belonging to a reserved set of clinically associated SNPs in dbSNP.

patient OS or PFS (supplemental Figure 1). Of the patients with WT-CDS only, those without nSNVs (n = 136) seemed to have poorer OS and PFS than those with nSNVs (n = 59), but the difference was not statistically significant ($P = .15$ for OS; $P = .17$ for PFS, respectively; Figure 3C-D). When 5-year survival rates were calculated, nSNVs conferred better survival in patients with WT-CDS (78% vs 59%, $P = .025$), and 5-year PFS also showed a trend (76% vs 58%, $P = .079$). In contrast, patients with MUT-CDS and nSNVs (n = 17) had poorer OS and PFS than did patients with MUT-CDS and no nSNVs (n = 32; $P = .019$ for OS; $P = .018$ for PFS; Figure 3E-F). Indeed, all patients with MUT-CDS and nSNVs died within 76 months after diagnosis (Figure 3E). We also combined SNPs and nSNVs as total SNVs; there were similar survival trends, but they were not statistically significant (supplemental Figure 2). After the cohort was divided into ABC- and GCB-DLBCL subsets, the prognostic impact of nSNVs in OS seemed to favor patients with WT-CDS and not favor patients with MUT-CDS in the GCB subset. Yet none were statistically significantly, most likely due to a smaller cohort (supplemental Figure 3). Again, nSNVs conferred better 5-year survival in GCB patients with WT-CDS ($P = .0006$). We did not find statistical differences in frequencies of subtypes, CDS mutation, and *TP53* monoallelic deletion between patients with nSNVs and those without.

Validation of *TP53* 3′UTR variants

To determine whether nSNVs are common in DLBCL, we sequenced the *TP53* 3′UTR in tumor samples from an additional 247 DLBCL patients. We found 106 nSNVs in 70 (28%) patients with 5 occurring twice. All 6 SNPs identified in the initial set were found in this 247 patient group (Table 1; supplemental Table 3). For the combined 491 patients, 187 nSNVs were identified in the *TP53* 3′UTR: 151 occurred once, 31 occurred twice, and 5 occurred 3 times (supplemental Table 4). These data indicate that nSNVs in the *TP53* 3′UTR occur frequently in DLBCL. In addition, nSNVs were dispersed across the whole *TP53* 3′UTR (Figure 4A), providing additional evidence that 3′UTR nSNVs are distributed more evenly than are CDS mutations.

3′UTR variants in miRNA-binding sites

Eleven miRNAs are reported to target the human *TP53* gene: miR-25, miR-30d,[20] miR-125b,[21] miR-504,[22] miR-380-5p,[23] miR-92a, miR-141,[24] miR-200a,[25] miR-1285,[26] miR-15a, and miR-16.[27] Among these miRNAs, 3 pairs have the same seed sequence: miR-25 and miR-92a, miR-141 and miR-200a, and miR-15a and miR-16. In addition, miR-504 and miR-1285 target 2 sites each. Therefore, the *TP53* 3′UTR has a total of 10 experimentally validated miRNA-targeting sites.

From www.bloodjournal.org by guest on February 13, 2017. For personal use only.

BLOOD, 30 MAY 2013 · VOLUME 121, NUMBER 22   *TP53* 3′UTR VARIATION IN DLBCL   4533
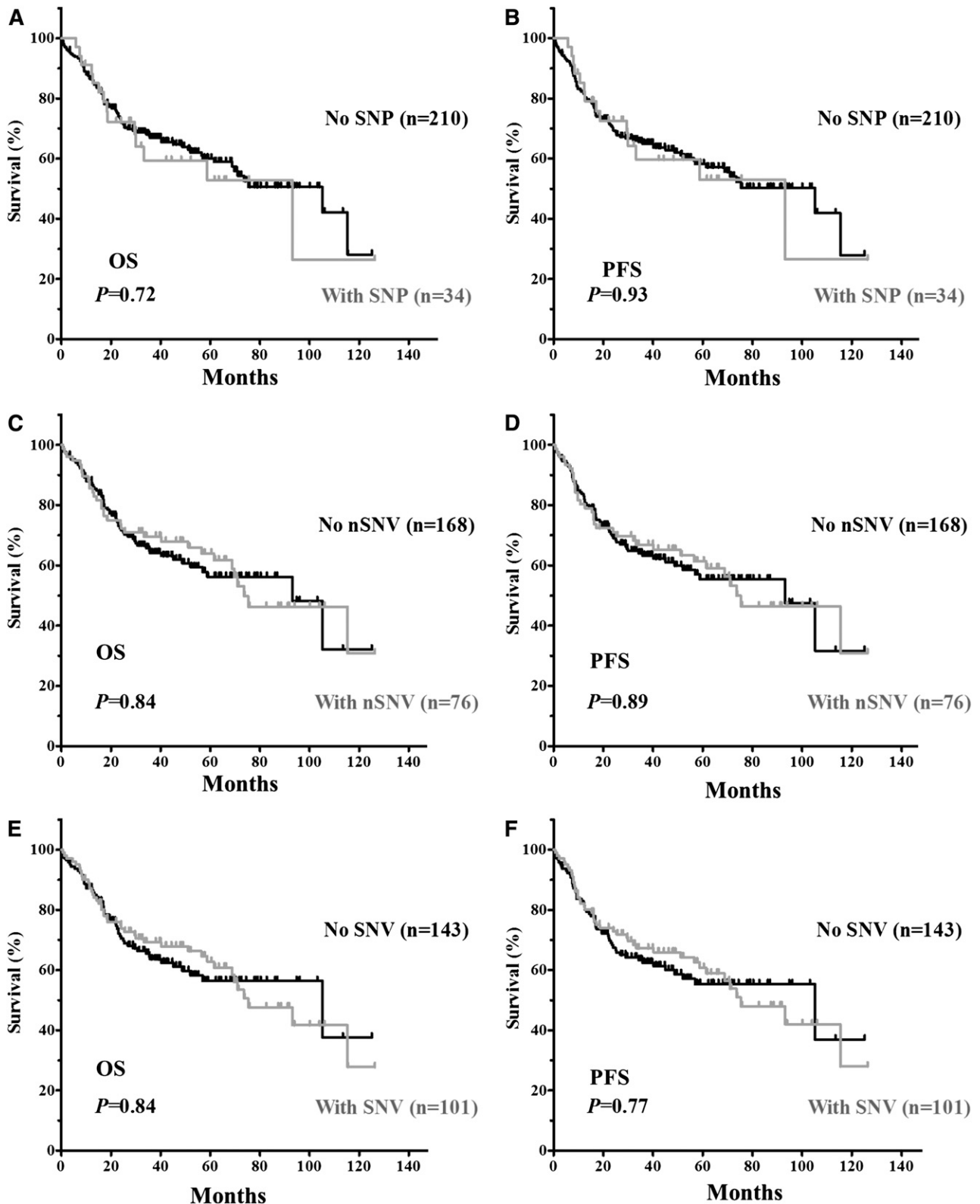
Figure 2. Kaplan-Meier estimates of OS and PFS in DLBCL patients with SNVs in the *TP53* 3′UTR. (A) OS of patients with or without SNPs. (B) PFS of patients with or without SNPs. (C) OS of patients with or without nSNVs. (D) PFS of patients with or without nSNV. (E) OS of patients with or without SNVs (either SNP or nSNV). (F) PFS of patients with or without SNVs.

Except for the miR-141 and miR-380-5p sites, 8 of 10 miRNA sites had 1 or more nSNVs occurring at least once in 491 patients (Table 2). We analyzed the potential of nSNVs to affect putative miRNA binding as predicted by TargetScan,[28,29] in which 8 nucleotides in the target 3′UTR are critical to miRNA suppression: a sequence complementary to the 6-nucleotide seed sequence of the miRNAs and 2 flanking nucleotides (A1 and m8). Of the 146 patients carrying 1 or more nSNVs in the 3′UTR, 95 (65%) had
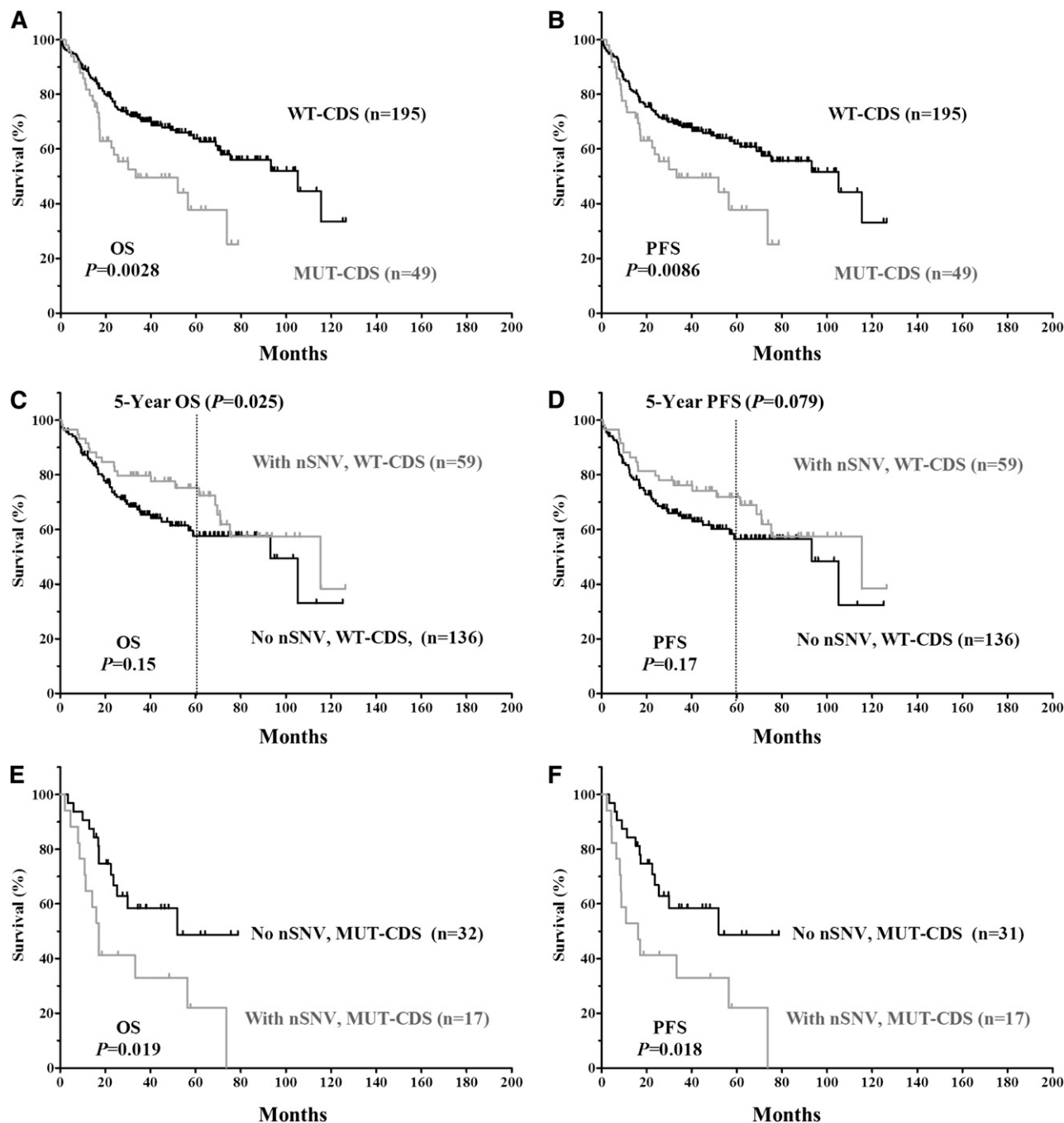
Figure 3. Kaplan-Meier estimates of OS and PFS in DLBCL patients classified according to *TP53* CDS status. (A) OS of patients with or without an MUT-CDS. (B) PFS of patients with or without an MUT-CDS. (C) OS of patients with a WT-CDS and nSNV. (D) PFS of patients with a WT-CDS and nSNVs. (E) OS of patients with an MUT-CDS and nSNV. (F) PFS of patients with MUT-CDS and nSNVs.

nSNVs that disrupt and/or generate miRNA-binding sites as predicted by TargetScan; 78 of 95 patients carried nSNVs disrupting putative miRNA binding (Table 3; Figure 4B).

p53 expression analysis of 3′UTR nSNVs

To test whether 3′UTR variation impacts p53 expression, we introduced gene expression cassettes carrying human *TP53* complementary DNA (cDNA) with 3′UTR variants in the miR-125b–binding site (Figure 5A) into p53-null H1299 cells. As shown in Figure 5B, *TP53* 3′UTR nSNVs did not alter p53 expression compared with

WT. However, when cells were cotransfected with miR-125b, 3 nSNVs (733U, 737A, and 739A) disrupting the seed match between miR-125b and the *TP53* 3′UTR released the negative regulation of p53 by miR-125b (Figure 5C). One variant (740A) that converts the seed match from 7mer to 8mer, which is predicted to have a greater effect on p53 downregulation, did not enhance p53 suppression by miR-125b. These results demonstrate that miRNAs and nSNVs in the *TP53* 3′UTR significantly alter p53 protein levels in vitro. We next analyzed the relationship between p53 protein expression with a 50% cutoff[17] and 3′UTR nSNVs in the initiation set. We found that there was no correlation between nSNVs and p53 expression in
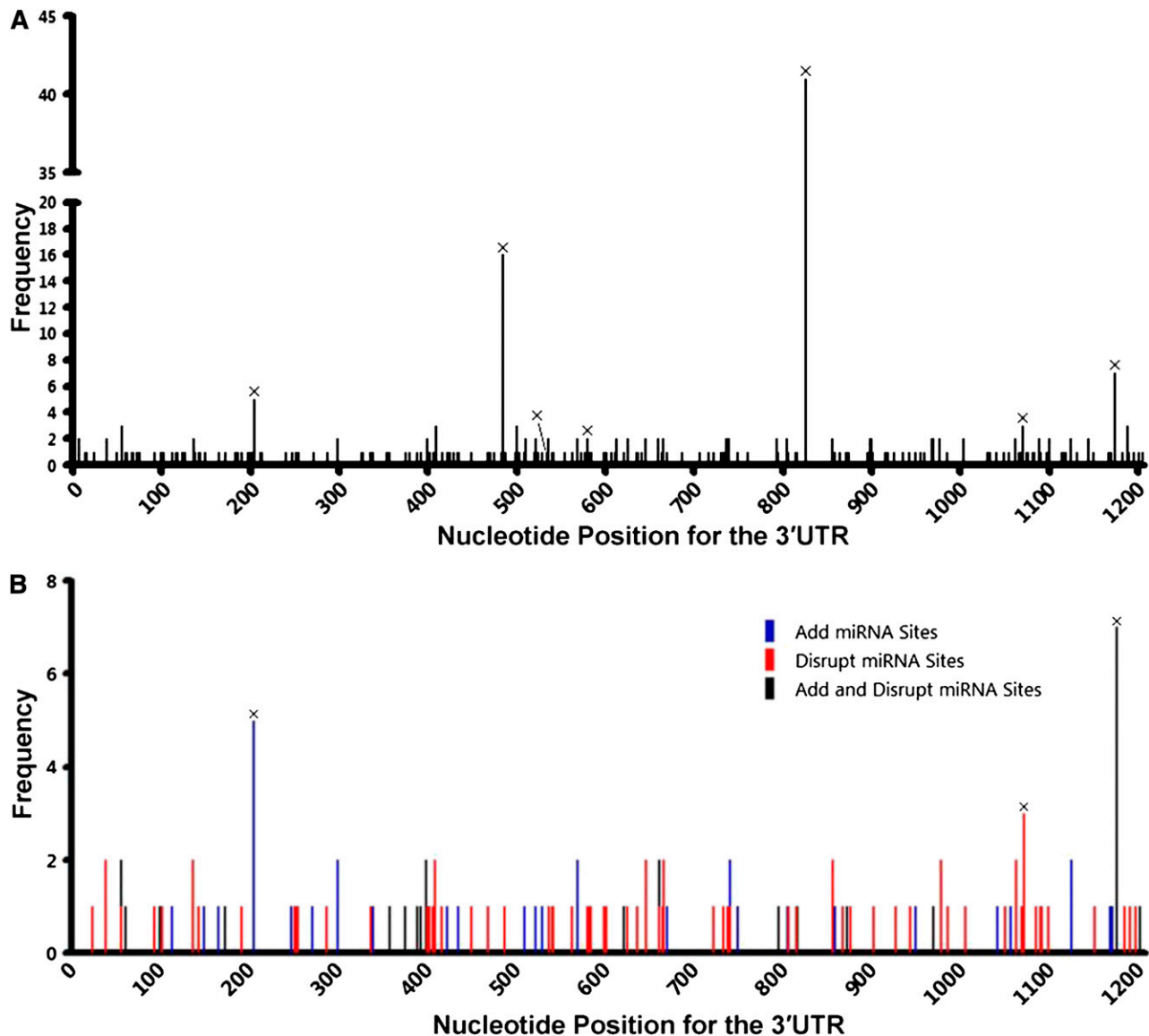
Figure 4. Variation in the *TP53* 3′UTR in 491 de novo DLBCL patients. (A) Distribution of SNVs in the 3′UTR. (B) Potential effects of SNVs on the interaction of p53 mRNA and miRNAs that are experimentally validated or predicted (by TargetScan) to target the *TP53* 3′UTR. SNVs listed in dbSNP are marked with ×.

the entire cohort, the WT-CDS group, or the MUT-CDS group (supplemental Table 5). There are at least 2 possibilities: (1) nSNVs may have either inhibitory or stimulatory effects on p53 regulation; and (2) the standard assay of p53 expression in tumors (percentage of cells with positive staining rather the signal strength)[17] cannot detect the subtle modulation of p53 protein levels by nSNVs.

Functional significance of a germline polymorphism in the *TP53* 3′UTR

SNP rs78378222 (A^{1175}C) is located in the polyadenylation signal (PAS) of *TP53* and is strongly associated with basal cell carcinoma (BCC).[30] This germline variant was found in 7 patients (6 A/C and 1 C/C). We predicted a decrease in p53 expression when the *TP53* gene contains the PAS variant (AATAAA>AATACA). We introduced gene expression cassettes carrying human *TP53* cDNA with this PAS variant (TP53ExtA^{1175}C), or a control SNP rs114831472 (TP53ExtC^{1170}T, not associated with BCC and other cancers) located 1 nucleotide upstream of the PAS, and the 2.5-kb genomic sequence downstream of the 3′UTR (Figure 6A) into p53-null

H1299 cells. We found significantly lower p53 protein levels in cells with TP53ExtA^{1175}C than in cells with WT control or TP53ExtC^{1170}T (Figure 6B). Northern blot analysis confirmed that p53 messenger RNA (mRNA) levels were downregulated in cells with the p53 PAS variant (Figure 6C). We assessed the effect of the *TP53* PAS variant on apoptosis by using the terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay. In cells harboring the p53 PAS minor allele, cellular apoptosis was significantly lower than that in cells expressing the WT *TP53* gene (Figure 6D). These data indicate that the *TP53* variant rs78378222 hinders p53 expression and its downstream functions in apoptosis.

Discussion

In this work, we found many previously unreported SNVs in the *TP53* 3′UTR in DLBCL tumor DNA samples. 3′UTR shortening[31]

Table 2. SNVs in the 3′UTR disrupting the validated seed match of *TP53*:miRNA

TP53 3′UTR and miRNA†	Consequential pairing†	Seed match‡	No. of patients*	
			Seed + m8 + 1A	Other
Pos. 93-98	5′ CUUUGAACCCUUGCU--**UGCAAU**A	7mer-1A	1(1)	0
	|∶∶ ||| ||||||			
hsa-miR-25/92a	3′ AGUCUGGCUCUGUUC**ACGUUA**C			
	|x||||			
nSNV 93G>A	5′ CUUUGAACCCUUGCU--**UA**C**AAU**A	NO		
Pos. 287-292	5′ UGCAGUUAAGGGUUA**GUUUAC**A	7mer-1A	1(1)	0
	|||| |||||||			
hsa-miR-30d	3′ GAAGGUCAGCCCCUA--**CAAAUG**U			
	|||| x||||||			
nSNV 287G>A	5′ UGCAGUUAAGGGUUA**A**U**UUAC**A	NO		
Pos.734-739	5′ AAGACUUGUUUUAUGC**UCAGGG**U	7mer-m8	6(4)	3(2)
	||| |||||||			
hsa-miR-125b	3′ AGUGUUCAAUCCCAG**AGUCCC**U			
	||| x|||x|xx			
nSNV 733C>T	5′ AAGACUUGUUUUAUG**U**U**CAGGG**U	NO		
nSNV 737G>C	5′ AAGACUUGUUUUAUGC**UCAC**G**G**U	NO		
nSNV 737G>A	5′ AAGACUUGUUUUAUGC**UCAA**G**G**U	NO		
nSNV 739G>A	5′ AAGACUUGUUUUAUGC**UCAGGA**U	NO		
nSNV 739G>C	5′ AAGACUUGUUUUAUGC**UCAGGC**U	NO		
nSNV 740U>A	5′ AAGACUUGUUUUAUGC**UCAGGG**A	8mer		
Pos. 736-741	5′ GACUUGUUUUAUGCUC**AGGGUC**A	8mer	6(5)	0
	|||||||			
hsa-miR-504	3′ CUAUCUCACGUCUGG**UCCCAG**A			
	||x|xx|			
nSNV 737G>C	5′ GACUUGUUUUAUGCUC**AAGGUC**A	NO		
nSNV 737G>A	5′ GACUUGUUUUAUGCUC**ACGGUC**A	NO		
nSNV 739G>A	5′ GACUUGUUUUAUGCUC**AGGAUC**A	NO		
nSNV 739G>C	5′ GACUUGUUUUAUGCUC**AGGCUC**A	NO		
nSNV 740U>A	5′ GACUUGUUUUAUGCUC**AGGGCC**A	NO		
Pos. 804-809	5′ UGGGUCUCGCUUUGUUG**CCCAG**G	7mer-m8	3(2)	4(3)
	∶||||||∶||||||||||			
hsa-miR-1285	3′ UCCAGAGUGAAACAAC**GGGUC**U			
	∶||||||∶||||||||xx|||			
nSNV 805C>T	5′ UGGGUCUCGCUUUGUUG**TCCAG**G	NO		
nSNV 806C>T	5′ UGGGUCUCGCUUUGUUG**CTCAG**G	NO		
Pos. 967-972	5′ GGGGUCUCACAGUGUU**GCCCAG**G	7mer-m8	5(2)	2(2)
	∶||||||||| |||||||			
hsa-miR-1285	3′ UCCAGAGUGAAACAAC**GGGUC**U			
	∶||||||||| |||||||			
nSNV 968C>T	5′ GGGGUCUCACAGUGUUG**TCCAG**G	NO		
nSNV 969C>T	5′ GGGGUCUCACAGUGUUG**CTCAG**G	NO		
Pos. 1065-1070	5′ CCACGUCCAGCUGGA**AGGGUC**A	7mer-1A	4(3)	1(1)
	||||||			
hsa-miR-504	3′ CUAUCUCACGUCUGG**UCCCAG**A			
	|x|x|x			
nSNV 1066G>A	5′ CCACGUCCAGCUGGA**A**A**GGUC**A	NO		
nSNV 1068G>A	5′ CCACGUCCAGCUGGA**AGGA**U**C**A	NO		
SNP 1070C>T	5′ CCACGUCCAGCUGGA**AGGGU**UA	NO		
nSNV 1071A>G	5′ CCACGUCCAGCUGGA**AGGGUC**G	6mer		
Pos. 1084-1089	5′ GUCAACAUCUUUUACAUU**CUGCA**A	?	3(2)	6(5)
	|| | ||| | |||| |			
hsa-miR-15	3′ GUGUUUGGUAAUACA**CGACGA**U			
hsa-miR-16	3′ GCGGUUAUAAAUGCA**CGACGA**U			
	| || ||||| | |||| |			
nSNV 1085C>T	5′ GUCAACAUCUUUUACAUU**CUGUA**A			
nSNV 1086A>G	5′ GUCAACAUCUUUUACAUU**CUGCG**A			

   *Number of patients carrying SNVs affecting putative miRNA binding. Unique SNVs are indicated in parentheses. SNVs affecting TP53:miRNA complementarity other than the seed match are not included. Underlining in the column 2 indicates the altered nucleotides.

   †Nucleotides in the *TP53* 3′ UTR are numbered starting from the one after the stop codon. Nucleotides complementary to the seed sequence (positions 2-7) of miRNAs are indicated. Position (pos.) numbers correspond to nucleotides that are complementary to the miRNA seed sequence (in bold). rs114831472, affecting miR-504 binding, is "SNP 1070C.T." One miR-504 site overlaps with the miR-125b site. "x" indicates that base pairing is disrupted.

   ‡The 7mer-m8 site comprises the seed match supplemented by a Watson-Crick match to miRNA nucleotide 8. The 7mer-A1 site comprises the seed match supplemented by an A across from miRNA nucleotide 1. The 8mer site comprises the seed match supplemented by both m8 and A1. "NO" indicates that the seed match was abolished. "?" indicates unconventional interaction.

Table 3. Occurrence of nSNVs in the *TP53* 3′UTR potentially affecting miRNA binding in DLBCL

	No. of patients (%)		
Subgroup	Training Set 76 of 244	Validation Set 70 of 247	Total 146 of 491
***TP53* CDS status**			
MUT-CDS	16 (21)	NA	NA
WT-CDS	60 (79)	NA	NA
3′UTR nSNV affecting putative miRNA binding*			
Generating only	12	5	17
Disrupting only	31	23	54
Generating and disrupting	15	9	24
Others	18	33	51
No. of 3′UTR nucleotides altered			
1	42 (59.5)	42 (60)	84 (57.5)
2	19 (23.8)	18 (25.7)	37 (25.3)
3	8 (9.5)	7 (10)	15 (10.3)
4	5 (4.8)	3 (4.3)	8 (5.5)
5	1 (1.2)	0	1 (0.7)
6	1 (1.2)	0	1 (0.7)

Others, outside seed match or not in miRNA sites; NA, not applicable.

*nSNVs predicted by TargetScan 5.0 to be targeted by miRNAs (based on the seed sequence of the miRNA plus A1 and m8). Some patients had both mutations generating miRNA binding and mutations disrupting it. Three SNPs listed in dbSNP that putatively affect miRNA binding (rs16956880 [205 G>A], rs11483 1472 [1070 G>A], and rs78378222 [1175 C>A]) were excluded.

and dysregulation of 3′UTR-binding proteins[32] or miRNAs[33] in cancer have been reported. This study, however, is the first to demonstrate that 3′UTR variation is widespread in the *TP53* gene in cancer patients. Most DLBCLs originate from B cells in the germinal center, which have a high proliferation rate and are prone to genomic alterations.[34] Germinal center B cells expand clonally and undergo physiological somatic hypermutation of their immunoglobulin variable-region genes and class-switch recombination to alter the immunoglobulin subtype mediated by activation-induced cytidine deaminase as part of the immune response. *BCL2* point mutations in DLBCL are suspected to be caused by this somatic hypermutation mechanism.[13] *TP53* 3′UTR nSNVs found in this study are predominantly G→A and C→T substitutions, and the frequency ratio of transition over transversion is 16.7, significantly higher than 2.1 (for all human variants in dbSNP), 2.0 (*TP53* CDS mutation in DLBCL[17]), or 7.3 (known SNPs of *TP53* 3′UTR). However, only a small number of nSNVs are located in WRCY/RGYW sites (IUPAC degenerated codes; supplemental Table 6), hot spots for physiological hypermutations mediated by activation-induced cytidine deaminase. In addition, these nSNVs are not found in many projects that clearly sequence the full *TP53* 3′UTR from hundreds of individuals.[35,36] Therefore, most nSNVs found in this study are likely cancer-specific mutations rather than germline polymorphisms or a result of a normally operative somatic hypermutation process. We acknowledge that the absence of patient-matched noncancerous tissue samples prevents us from completely ruling out the possibility of rare private germline variants in these patients. Counting CDS mutations and 3′UTR nSNVs, ~45% of DLBCL patients (109 of 244) had alterations in *TP53*, making *TP53* the most frequently mutated gene identified in DLBCL. A recent study reported an *R*-CHOP treatment-associated pattern of p53 and cell cycle dysregulation in DLBCL,[37] further emphasizing the importance of p53 in DLBCL pathogenesis and prognosis.

Most of the *TP53* 3′UTR nSNVs are located in validated or putative miRNA-binding sites. Each specific mammalian cell

expresses ~70 miRNAs at an appreciable level,[38] yet normal B-cell development and the initiation of malignancy and tumor progression are ordered and dynamic processes in which hundreds of miRNAs are differentially expressed in more than a dozen distinct cell populations.[39-42] Thus, 3′UTR variation may have a direct biological impact on potential interactions between miRNAs and p53 in B-cell maturation or DLBCL pathogenesis. Because miRNAs repress gene expression, 3′UTR alterations that are predicted to disrupt miRNA-binding sites, as occurred in 78 of 146 patients with 1 or more nSNVs in this study (Table 3), could reduce the efficiency of miRNA suppression and subsequently increase p53 expression. This has been experimentally validated for a few nSNVs located at the miR-125b site. We reasoned that when the *TP53* CDS is WT, the increased levels of tumor-suppressive p53 protein confer better 5-year survival in patients with 3′UTR nSNVs, as WT p53 is advantageous for patients undergoing *R*-CHOP chemotherapy[17]; in contrast, when the *TP53* CDS is mutated concomitantly, the elevated levels of oncogenic p53 will worsen survival in patients with 3′UTR nSNVs. Our findings do show that variation in the *TP53* 3′UTR as a prognostic indicator of survival in DLBCL patients is dependent on the status of the CDS, yet there is no correlation between nSNV and p53 protein expression in the entire cohort, the WT-CDS group, or the MUT-CDS group. It should be noted that 3′UTR variation may also disrupt interactions between p53 mRNA and RNA-binding proteins, although the exact nucleotides have not been pinpointed.[43,44] Additionally, 3′UTR variation could affect mRNA stability or translation efficiency due
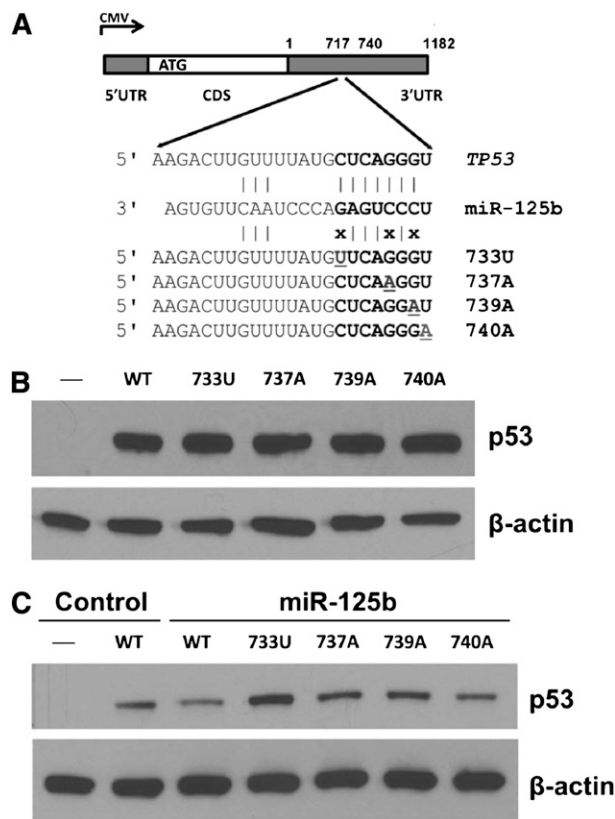

Figure 5. Expression analyses of *TP53* 3′UTR variation affecting miR-125b binding in p53-null cells. (A) Schematic representation of the p53 cDNA construct and 3′UTR variants. (B) Western blot of p53 expression in p53-null H1299 cells transfected with p53 constructs. (C) Western blot of p53 expression in p53-null H1299 cells transfected with p53 constructs with or without miR-125b. β-actin was used as a control.
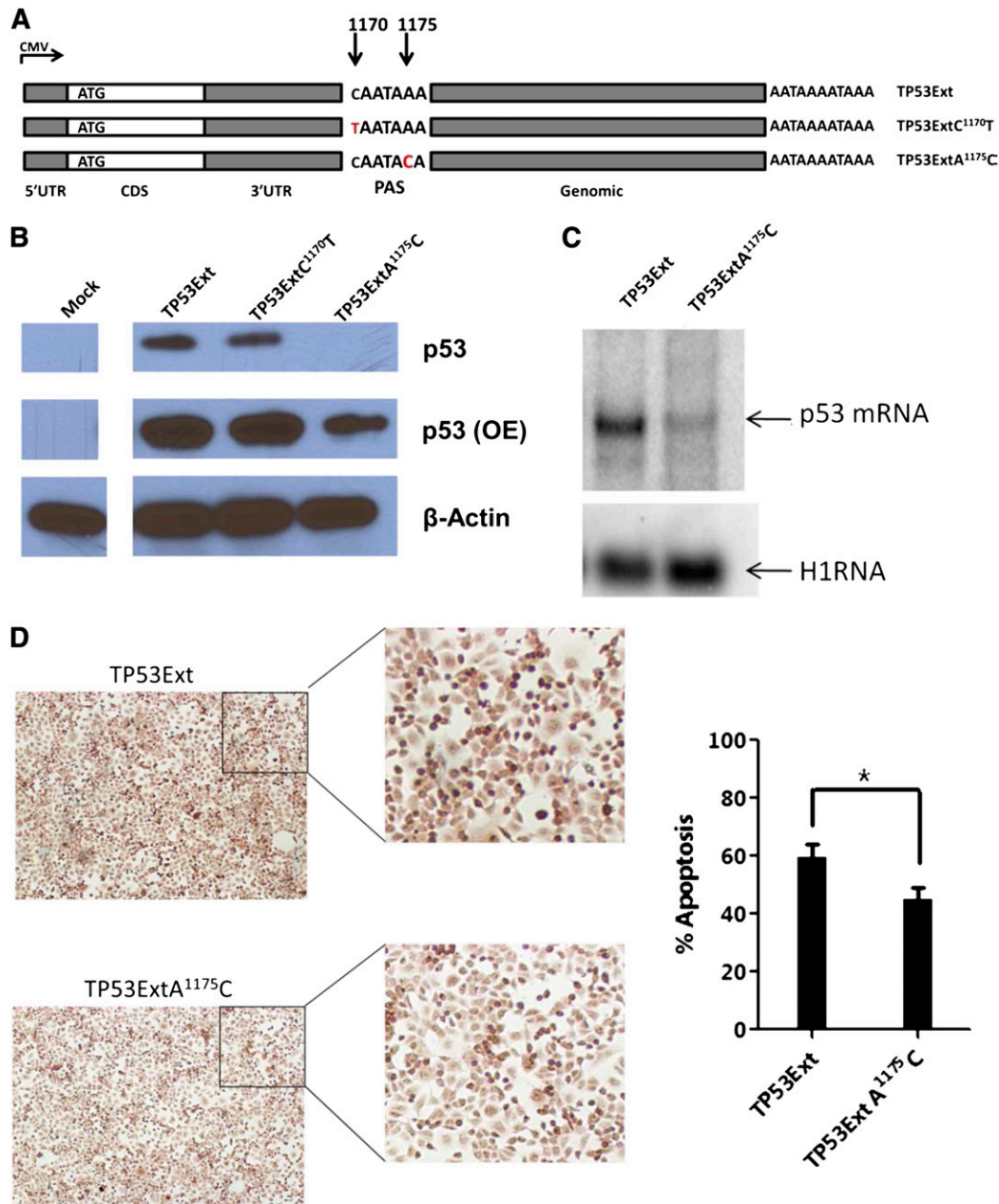
Figure 6. p53 expression and function for the *TP53* PAS variant in p53-null cells. (A) Schematic representation of the *TP53* constructs. Each carries a WT 5′UTR, WT CDS, a WT or mutant 3′UTR, and a 2.5-kb native genomic sequence downstream of the *TP53* PAS. (B) Western blot of p53 expression in p53-null H1299 cells transfected with p53 constructs. β-actin was used as a control. OE, overexposure. (C) Northern blot of p53 mRNAs in H1299 cells carrying exogenous p53 genes. A housekeeping RNA (H1RNA, the RNA subunit of human ribonuclease P) was used as a control. (D) Apoptosis of cells with p53 constructs; representative TUNEL images (left) and percentage of TUNEL-positive cells (right). *$P \leq .05$.

to other mechanisms. Further investigation is needed to ascertain the role of the nSNVs in p53 regulation and DLBCL prognosis.

The disappearance of the survival advantage at ~10 years for DLBCL patients with a WT-CDS and nSNVs is noteworthy. The frequency of *TP53* CDS mutations may be higher in relapsed DLBCL patients, and this may partially override the impact of 3′UTR variation on survival beyond 5 years. In patients with multiple myeloma, the frequency of *TP53* CDS mutations in de novo disease is only ~3%, yet this number rises up to 30% in patients with relapsed disease.[45] Relapsed DLBCL is generally diagnosed by imaging and limited sampling such as fine-needle aspiration biopsy rather than by excisional biopsy; the lack of adequate specimens for

genetic analysis precluded our testing for *TP53* CDS mutations in DLBCL patients at relapse and is a limitation of this study.

TP53 germline variants have been investigated for cancer susceptibility using genome-wide association studies, in which a type I error rate (*P*-value threshold for significance) of 10^{-7} is generally regarded as indicating genome-wide significance.[3,46] Until 2011, no genome-wide association study reported a significant association between any germline *TP53* polymorphism (other than Li-Fraumeni Syndrome variants) and any cancer with a *P* value $\leq 10^{-7}$. In 2011, however, rs78378222 was found to be strongly associated with BCC, with an odds ratio (OR) = 2.16 ($P = 2.2 \times 10^{-20}$).[36] rs78378222 is located in the fifth nucleotide of the *TP53* PAS that is required for

polyadenylation machinery recognition and subsequent cleavage, polyadenylation, and export of mature mRNAs to the cytoplasm. The risk-associated allele is a "C," resulting in an alternative PAS (AATACA) replacing the canonical PAS (AATAAA). Among millions of SNPs in the human genome, rs78378222 has the strongest association with BCC.[36] This SNP is also associated with an increased risk of prostate cancer (OR = 1.44; $P = 2.4 \times 10^{-6}$), glioma (OR = 2.35; $P = 1.0 \times 10^{-5}$), and colorectal adenoma (OR = 1.39; $P = 1.6 \times 10^{-4}$),[36] as well as esophageal squamous cell carcinoma (OR = 3.22; $P = 1.34 \times 10^{-4}$).[47] In an independent confirmation study, rs78378222 was found to be associated with a 3.5 times higher risk of glioma ($P = 1.34 \times 10^{-4}$).[48] Stefansson and colleagues showed that rs78378222[A/C] heterozygotes expressed "somewhat less" p53 transcript levels than WT homozygotes in human blood specimens ($P = .041$).[36] In the present study, we tested the role of the alternative PAS in p53 regulation using a p53-null cell line with exogenous p53 constructs. We found that the C allele, as compared with the A allele, dramatically lowers p53 mRNA levels and results in reduced p53 protein expression and cellular apoptosis. Our data are in agreement with a previous report that simian virus 40 mRNA with AATACA as PAS exhibits an eightfold decrease in cleavage efficiency compared with the canonical site.[49,50] These findings provide direct evidence that rs78378222 negatively impacts p53 expression and function. p53 stabilization and function rely on various posttranslational modifications. As rs78378222 occurs in the PAS signal of TP53, none of the downstream p53 protein modifications are affected. Thus, this variant presents a unique instance of TP53 alleles with unambiguous reduced function, in contrast with most CDS mutants that are associated with a "loss of function" and/or a "gain of function."[5]

In summary, in this study we have shown that sequence variation in the TP53 3'UTR is frequent in DLBCL and that the role of this sequence variation in patient survival depends on the status of the TP53 CDS. To our knowledge, this study is the first to demonstrate the prognostic importance of the TP53 3'UTR in cancer or any other disease. The full extent of the pathobiology of 3'UTR variants remains to be determined.

Acknowledgments

Authorship

Contribution: Y.L., K.H.Y., Z.Y.X.-M., and M.W.G. designed and conducted the research, performed and supported the statistical analysis, and wrote the manuscript; Y.L., Z.Y.X.-M., L.W., C.V., A.T., D.Z., L.Q., S.M.-M., K.D., A.C., Y.Z., G.B., K.L.R., E.D.H., W.W.L.C., J.H.v.K., Q.H., W.A., M.P., A.J.M.F., J.N.W., R.S.G., M.A.P., M.B.M., L.W., M.W., K.S.R., L.J.M., and K.H.Y. contributed vital new reagents, resources, and analytical tools under approval by the institutional review boards and the material transfer agreement; Z.Y.X.-M., C.V., A.T., S.M.-M., K.D., A.O., Y.Z., G.B., K.L.R., E.D.H., W.W.L.C., J.H.v.K., Q.H., W.A., M.P., A.J.M.F., J.N.W., R.S.G., M.A.P., M.B.M., M.W., L.J.M., and K.H.Y. collected clinical and follow-up data under approval by the institutional review boards and the material transfer agreement; Y.L., Z.Y.X.-M., C.V., and K.H.Y. analyzed the data and performed the statistical analysis; and all authors contributed vital strategies, participated in discussions, and provided scientific input.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence: Yong Li, Department of Biochemistry and Molecular Biology, School of Medicine, University of Louisville, 319 Abraham Flexner Way, Louisville, KY 40202; e-mail: yong.li@louisville.edu; and Ken H. Young, Department of Hematopathology, Unit 72, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX 77230-1439; e-mail: khyoung@mdanderson.org.

References

1. Ventura A, Kirsch DG, McLaughlin ME, et al. Restoration of p53 function leads to tumour regression in vivo. Nature. 2007;445(7128):661-665.

2. Xue W, Zender L, Miething C, et al. Senescence and tumour clearance is triggered by p53 restoration in murine liver carcinomas. Nature. 2007;445(7128):656-660.

3. Whibley C, Pharoah PDP, Hollstein M. p53 polymorphisms: cancer implications. Nat Rev Cancer. 2009;9(2):95-107.

4. Palmero EI, Achatz MI, Ashton-Prolla P, Olivier M, Hainaut P. Tumor protein 53 mutations and inherited cancer: beyond Li-Fraumeni syndrome. Curr Opin Oncol. 2010;22(1):64-69.

5. Oren M, Rotter V. Mutant p53 gain-of-function in cancer. Cold Spring Harb Perspect Biol. 2010;2(2):a001107.

6. Olive KP, Tuveson DA, Ruhe ZC, et al. Mutant p53 gain of function in two mouse models of Li-Fraumeni syndrome. Cell. 2004;119(6):847-860.

7. Lang GA, Iwakuma T, Suh YA, et al. Gain of function of a p53 hot spot mutation in a mouse model of Li-Fraumeni syndrome. Cell. 2004;119(6):861-872.

8. Storey A, Thomas M, Kalita A, et al. Role of a p53 polymorphism in the development of human papillomavirus-associated cancer. Nature. 1998;393(6682):229-234.

9. Lenz G, Staudt LM. Aggressive lymphomas. N Engl J Med. 2010;362(15):1417-1429.

10. Nogai H, Dörken B, Lenz G. Pathogenesis of non-Hodgkin's lymphoma. J Clin Oncol. 2011;29(14):1803-1811.

11. Pasqualucci L, Trifonov V, Fabbri G, et al. Analysis of the coding genome of diffuse large B-cell lymphoma. Nat Genet. 2011;43(9):830-837.

12. Morin RD, Mendez-Lago M, Mungall AJ, et al. Frequent mutation of histone-modifying genes in non-Hodgkin lymphoma. Nature. 2011;476(7360):298-303.

13. Lohr JG, Stojanov P, Lawrence MS, et al. Discovery and prioritization of somatic mutations in diffuse large B-cell lymphoma (DLBCL) by whole-exome sequencing. Proc Natl Acad Sci USA. 2012;109(10):3879-3884.

14. Vogelstein B, Lane D, Levine AJ. Surfing the p53 network. Nature. 2000;408(6810):307-310.

15. Vogelstein B, Sur S, Prives C. p53: the most frequently altered gene in human cancers. Nature Education. 2010;3(9):6.

16. Young KH, Leroy K, Møller MB, et al. Structural profiles of TP53 gene mutations predict clinical outcome in diffuse large B-cell lymphoma: an international collaborative study. *Blood.* 2008; 112(8):3088-3098.

17. Xu-Monette ZY, Wu L, Visco C, et al. Mutational profile and prognostic significance of TP53 in diffuse large B-cell lymphoma patients treated with R-CHOP: report from an International DLBCL Rituximab-CHOP Consortium Program Study. *Blood.* 2012;120(19):3986-3996.

18. Lu Z, Li Y, Takwi A, et al. miR-301a as an NF-κB activator in pancreatic cancer cells. *EMBO J.* 2011;30(1):57-67.

19. Petitjean A, Mathe E, Kato S, et al. Impact of mutant p53 functional properties on TP53 mutation patterns and tumor phenotype: lessons from recent developments in the IARC TP53 database. *Hum Mutat.* 2007;28(6):622-629.

20. Kumar M, Lu Z, Takwi AAL, et al. Negative regulation of the tumor suppressor p53 gene by microRNAs. *Oncogene.* 2011;30(7):843-853.

21. Le MTN, Teh C, Shyh-Chang N, et al. MicroRNA-125b is a novel negative regulator of p53. *Genes Dev.* 2009;23(7):862-876.

22. Hu W, Chan CS, Wu R, et al. Negative regulation of tumor suppressor p53 by microRNA miR-504. *Mol Cell.* 2010;38(5):689-699.

23. Swarbrick A, Woods SL, Shaw A, et al. miR-380-5p represses p53 to control cellular survival and is associated with poor outcome in MYCN-amplified neuroblastoma. *Nat Med.* 2010;16(10):1134-1140.

24. Neveu P, Kye MJ, Qi S, et al. MicroRNA profiling reveals two distinct p53-related human pluripotent stem cell states. *Cell Stem Cell.* 2010;7(6): 671-681.

25. Becker LE, Lu Z, Chen W, Xiong W, Kong M, Li Y. A systematic screen reveals MicroRNA clusters that significantly regulate four major signaling pathways. *PLoS ONE.* 2012;7(11):e48474.

26. Tian S, Huang S, Wu S, Guo W, Li J, He X. MicroRNA-1285 inhibits the expression of p53 by directly targeting its 3′ untranslated region. *Biochem Biophys Res Commun.* 2010;396(2): 435-439.

27. Fabbri M, Bottoni A, Shimizu M, et al. Association of a microRNA/TP53 feedback circuitry with pathogenesis and outcome of B-cell chronic lymphocytic leukemia. *JAMA.* 2011;305(1):59-67.

28. Friedman RC, Farh KK-H, Burge CB, Bartel DP. Most mammalian mRNAs are conserved targets of microRNAs. *Genome Res.* 2009;19(1):92-105.

29. Bartel DP. MicroRNAs: target recognition and regulatory functions. *Cell.* 2009;136(2):215-233.

30. Stacey SN, Sulem P, Jonasdottir A, et al. A germline variant in the TP53 polyadenylation signal confers cancer susceptibility. *Nat Genet.* 2011;43(11):1098-1103.

31. Mayr C, Bartel DP. Widespread shortening of 3′UTRs by alternative cleavage and polyadenylation activates oncogenes in cancer cells. *Cell.* 2009;138(4):673-684.

32. Topisirovic I, Siddiqui N, Orolicki S, et al. Stability of eukaryotic translation initiation factor 4E mRNA is regulated by HuR, and this activity is dysregulated in cancer. *Mol Cell Biol.* 2009;29(5): 1152-1162.

33. Croce CM. Causes and consequences of microRNA dysregulation in cancer. *Nat Rev Genet.* 2009;10(10):704-714.

34. Klein U, Dalla-Favera R. Germinal centres: role in B-cell physiology and malignancy. *Nat Rev Immunol.* 2008;8(1):22-33.

35. Packer BR, Yeager M, Burdett L, et al. SNP500Cancer: a public resource for sequence validation, assay development, and frequency analysis for genetic variation in candidate genes. *Nucleic Acids Res.* 2006;34(Database issue suppl 1):D617-D621.

36. Stacey SN, Sulem P, Jonasdottir A, et al. Swedish Low-risk Colorectal Cancer Study Group. A germline variant in the TP53 polyadenylation signal confers cancer susceptibility. *Nat Genet.* 2011;43(11):1098-1103.

37. Monti S, Chapuy B, Takeyama K, et al. Integrative analysis reveals an outcome-associated and targetable pattern of p53 and cell cycle deregulation in diffuse large B cell lymphoma. *Cancer Cell.* 2012;22(3):359-372.

38. Landgraf P, Rusu M, Sheridan R, et al. A mammalian microRNA expression atlas based on small RNA library sequencing. *Cell.* 2007;129(7): 1401-1414.

39. Petriv OI, Kuchenbauer F, Delaney AD, et al. Comprehensive microRNA expression profiling of the hematopoietic hierarchy. *Proc Natl Acad Sci USA.* 2010;107(35):15443-15448.

40. Spierings DC, McGoldrick D, Hamilton-Easton AM, et al. Ordered progression of stage-specific miRNA profiles in the mouse B2 B-cell lineage. *Blood.* 2011; 117(20):5340-5349.

41. Zhang J, Jima DD, Jacobs C, et al. Patterns of microRNA expression characterize stages of human B-cell differentiation. *Blood.* 2009;113(19): 4586-4594.

42. Malumbres R, Sarosiek KA, Cubedo E, et al. Differentiation stage-specific expression of microRNAs in B lymphocytes and diffuse large B-cell lymphomas. *Blood.* 2009;113(16): 3754-3764.

43. Takagi M, Absalon MJ, McLure KG, Kastan MB. Regulation of p53 translation and induction after DNA damage by ribosomal protein L26 and nucleolin. *Cell.* 2005;123(1):49-63.

44. Mazan-Mamczarz K, Galbán S, López de Silanes I, et al. RNA-binding protein HuR enhances p53 translation in response to ultraviolet light irradiation. *Proc Natl Acad Sci USA.* 2003; 100(14):8354-8359.

45. Chng WJ. Genetic abnormalities in multiple myeloma. In: Kumar S, Abraham J, eds. *Multiple Myeloma.* New York, NY: Demos Medical Publishing; 2010:215-236.

46. Thomas DC, Haile RW, Duggan D. Recent developments in genomewide association scans: a workshop summary and review. *Am J Hum Genet.* 2005;77(3):337-345.

47. Zhou L, Yuan Q, Yang M. A functional germline variant in the P53 polyadenylation signal and risk of esophageal squamous cell carcinoma. *Gene.* 2012;506(2):295-297.

48. Egan KM, Nabors LB, Olson JJ, et al. Rare TP53 genetic variant associated with glioma risk and outcome. *J Med Genet.* 2012;49(7):420-421.

49. Wickens M, Stephenson P. Role of the conserved AAUAAA sequence: four AAUAAA point mutants prevent messenger RNA 3′ end formation. *Science.* 1984;226(4678):1045-1051.

50. Zarkower D, Stephenson P, Sheets M, Wickens M. The AAUAAA sequence is required both for cleavage and for polyadenylation of simian virus 40 pre-mRNA in vitro. *Mol Cell Biol.* 1986;6(7): 2317-2323.

Single nucleotide variation in the *TP53* 3′ untranslated region in diffuse large B-cell lymphoma treated with rituximab-CHOP: a report from the International DLBCL Rituximab-CHOP Consortium Program

Yong Li, Michael W. Gordon, Zijun Y. Xu-Monette, Carlo Visco, Alexander Tzankov, Dehui Zou, Lugui Qiu, Santiago Montes-Moreno, Karen Dybkaer, Attilio Orazi, Youli Zu, Govind Bhagat, Kristy L. Richards, Eric D. Hsi, William W. L. Choi, J. Han van Krieken, Qin Huang, Weiyun Ai, Maurilio Ponzoni, Andrés J. M. Ferreri, Jane N. Winter, Ronald S. Go, Miguel A. Piris, Michael B. Møller, Lin Wu, Michael Wang, Kenneth S. Ramos, L. Jeffrey Medeiros and Ken H. Young

Updated information and services can be found at:
http://www.bloodjournal.org/content/121/22/4529.full.html

Articles on similar topics can be found in the following Blood collections
Lymphoid Neoplasia (2461 articles)

Information about reproducing this article in parts or in its entirety may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#reprints

Information about subscriptions and ASH membership may be found online at:
http://www.bloodjournal.org/site/subscriptions/index.xhtml