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Loss of p73 promotes dissemination of Myc-induced B cell lymphomas in mice

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Mice engineered to express c-Myc in B cells (Eμ-myc mice) develop lethal lymphomas in which the gene encoding the p53 tumor suppressor is frequently mutated. Whether the p53 homolog p73 also functions as a tumor suppressor in vivo remains controversial. Here we have shown that p73 loss does not substantially affect disease onset and mortality in Eμ-myc mice. However, it does alter the phenotype of the disease. Specifically, p73 loss decreased nodal disease and increased widespread extranodal dissemination. We further found that p53 acted as the dominant tumor suppressor during the onset of Eμ-myc–driven B cell lymphomagenesis, while p73 modulated tumor dissemination and extranodal growth. Immunophenotyping and expression profiling suggested that p73 loss allowed increased maturation of malignant B cells and deregulated genes involved in lymphocyte homing and dissemination of human lymphomas. Consistent with this, p73 expression was frequently downregulated in a large cohort of human mature aggressive B cell lymphomas, and both the incidence and degree of p73 downregulation in these tumors correlated with their extranodal dissemination status. These data indicate that p73 is a modifier of Myc-driven lymphomas in mice, favoring tumor dissemination, and suggest that p73 could be a biomarker for human B cell lymphoma dissemination, a notion that can now be tested in clinicopathologic correlation studies.

Introduction

The *p73* gene was identified through its structural homology with the p53 tumor suppressor (1, 2). Initial overexpression studies demonstrated that p73 can activate many p53-responsive genes and partly substitute for p53 in triggering cell-cycle arrest or apoptosis (3–5). However, p73 and p53 are not functionally equivalent. Recent analyses revealed that p73 has its own set of transcriptional target genes (6, 7). Moreover, the *TP73* gene locus is complex and encodes 2 classes of isoforms with opposing activities. p73 variants that lack the N-terminal transactivation (TA) domain function to interfere with the activity of the full-length counterparts of p73, p53, and p63 (8, 9). A net upregulation of the dominant negative ΔNp73 isoforms (i.e., a high ΔN/TAp73 ratio) frequently occurs in carcinomas and is a poor prognostic marker in some solid tumors (9–11).

Although p53 and p73 share structural similarities, their differences in vivo are striking. Thus, p53-null mice are developmentally normal but show a high predisposition to spontaneous tumors, mainly T lymphomas. In contrast, in vivo evidence for a tumor-suppressor role of p73 is still scarce and contradictory. p73-knockout mice (which lack all p73 isoforms) are born with developmental neurological and immunological defects associated with early lethality, but show no tumor predisposition (12). Flores et al. (13) reported spontaneous development of mainly microscopic tumors in *p73+/−* and *p73−/−* mice, particularly when combined with p53 heterozygosity, while p73 did not contribute to p53 suppressor activity during irradiation-induced T cell lymphomagenesis (14). On the other hand, aging mice specifically lacking TAp73 develop spontaneous malignancies, mainly lung adenocarcinomas and lymphomas (15).

Unlike p53, the *p73* gene is rarely mutated in human cancers (10). In fact, there is currently no compelling genetic evidence that inactivation of p73 is required for malignant transformation or progression in human tumors. The single notable exception comes from persistent reports of frequent loss of p73 expression in hematological malignances. Specifically, inactivation of the *p73* gene by epigenetic silencing or deletion is a common finding in malignant lymphoproliferative disorders. p73 is inactivated in about 35% of acute lymphoblastic leukemia (ALL) and about one-third of non-Hodgkin lymphomas (NHLs) (16–19). Of note, 95% of human lymphomas are B cell in origin (20). Also, in a study of natural killer cell lymphomas, the *p73* gene was methylated in 94% of cases (21). Assessment of p73 methylation may provide important prognostic information, as shown in patients with ALL (19). In sum, these data suggest that p73 might play a pathogenetic role in human lymphomas.

To experimentally assess the role of p73 in B cell lymphomagenesis, we investigated Eμ-myc transgenic mice, the well-characterized model of human NHL. These mice overexpress c-Myc in B cell progenitors under the control of the immunoglobulin heavy chain enhancer and develop pre–B cell and/or B cell lymphomas (22, 23). The latent period prior to onset of disease reflects the ability of c-Myc to induce p53-dependent and p53-independent growth arrest and apoptotic checkpoints that protect animals against tumor formation. These checkpoints become disabled when overtly malignant cells emerge (24). Given the fact that a significant fraction of human B lymphomas exhibit deregulated c-Myc and p73 and that c-Myc activates proapoptotic functions of p73 in vitro (25), Eμ-myc mice provide a relevant in vivo model to assess the impact of p73 on tumor behavior.

Results

p73-deficient Eμ-myc mice are viable but show developmental defects. It was reported that *p73−/−* mice survive to birth, but most of them die within 2 months due to brain defects, chronic infections, and inflammation (12). Indeed, in mixed litter colonies generated
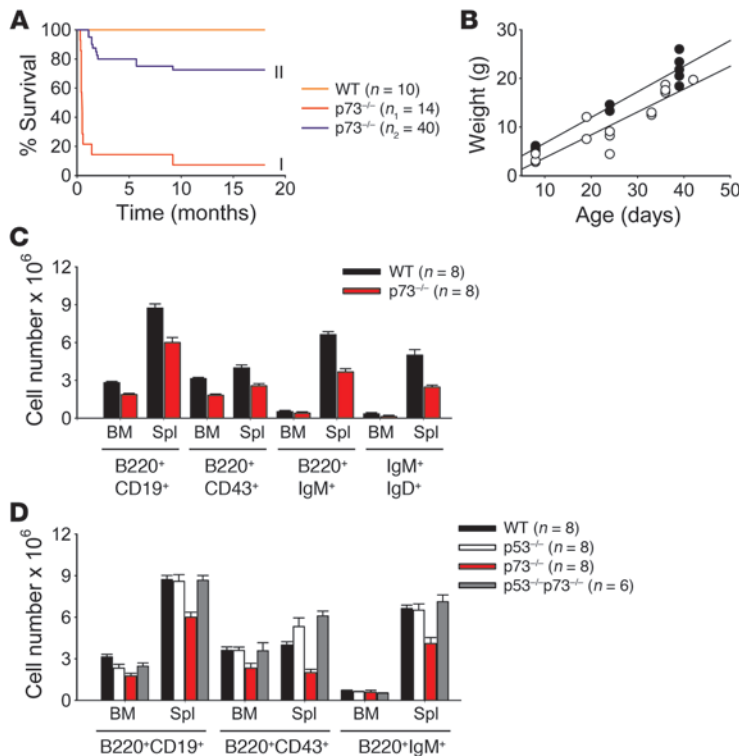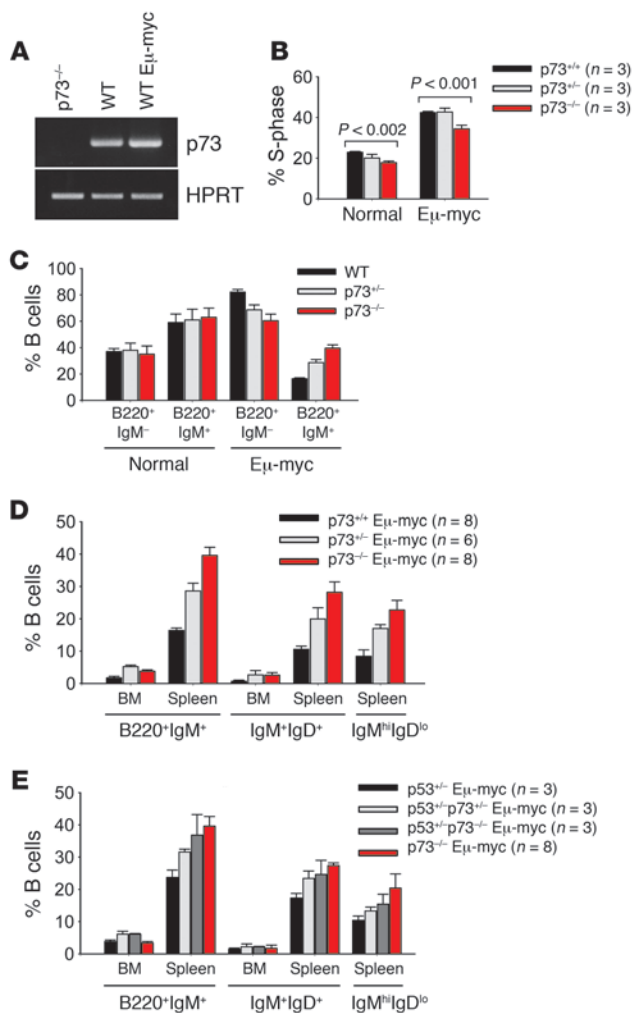
Figure 1
Mice deficient in p73 have impaired B cell development. (**A**) Overall survival of WT and *p73*⁻/⁻ mice before (curve I) and after (curve II) removal of intralitter competition from WT and *p73*⁺/⁻ siblings. The number of animals per genotype is indicated. Curve II: while a minority (25%) of *p73*⁻/⁻ mice die prematurely due to severe hydrocephalus with intraventricular hemorrhage, the remainder survive long term and remain tumor free. (**B**) Postpartum body weight gain of WT (black circles) and rescued *p73*⁻/⁻ mice (white circles) shown in Figure 1A, curve II. (**C**) Cells extracted from BM and spleens (Spl) of 4-week-old nontransgenic WT and *p73*⁻/⁻ mice were costained with antibodies to B220, CD19, CD43, IgM, and IgD and analyzed by FACS. Absolute numbers of total B cells (B220⁺CD19⁺), pro–B cells (B220⁺CD43⁺), immature/mature B cells (B220⁺IgM⁺), and recirculating B cells (IgM⁺IgD⁺) are graphed. Error bars represent SEM. (**D**) Cells extracted from BM and spleens of 4-week-old mice of the indicated genotypes were stained and analyzed as in **C**. Error bars represent SEM.

from interbreeding heterozygous animals, survival of *p73*⁻/⁻ mice decreased sharply to less than 20%, as previously described (Figure 1A) (12). However, we discovered that most *p73*⁻/⁻ animals die at this young age mainly because of malnutrition caused by their inability to compete with WT and *p73*⁺/⁻ littermates. Identifying such *p73*⁻/⁻ pups right after birth and moving them to lactating foster mothers essentially normalized their weight gain and rescued overall long-term longevity to nearly 80% (Figure 1, A and B). As reported (12), rescued *p73*⁻/⁻ mice developed variably penetrant congenital hydrocephalus, which killed 25% of them within 9 months due to intraventricular hemorrhage (Figure 1A). Infections, although present, were mild and did not cause death. Of note, none of the long-term *p73*⁻/⁻ survivors developed a malignancy. Thus far, we produced more than 40 *p73*⁻/⁻ Eμ-myc mice, of which 20% again displayed severe brain defects causing their premature death. Most of the remaining *p73*⁻/⁻ Eμ-myc mice exhibited nonfatal brain defects and developed B cell lymphomas (see below).

To determine whether p73 loss affects normal B cell development, BM and peripheral lymphoid organs (spleen, lymph nodes) of nontransgenic *p73*⁺/⁺, *p73*⁺/⁻, and *p73*⁻/⁻ mice were characterized by flow cytometry. At birth and through 8 weeks of age, lymphoid organs from *p73*⁺/⁻ and *p73*⁻/⁻ mice displayed no morphological defects and showed an overall normal proportional distribution of B cell subsets, including pro–B cells (B220⁺CD43⁺), immature/mature B cells (B220⁺IgM⁺), and recirculating B cells (IgM⁺IgD⁺) (Figure 1C and data not shown). However, the absolute cell number of each B cell subset in *p73*⁻/⁻ mice was decreased by 30%–40% compared with WT controls (Figure 1C). This B cell hypocellularity in *p73*⁻/⁻ mice is mediated by p53, since codeletion of p53 completely rescued B cellularity in *p73*⁻/⁻ mice. Accordingly, *p53*⁻/⁻*p73*⁻/⁻ (DKO) mice showed normal numbers of all major B cell subsets, most notably within the immature BM and mature

spleen B cell compartments (Figure 1D). Moreover, B cells from spleens of *p73*⁻/⁻ mice showed increased Puma levels compared with WT mice (Supplemental Figure 1C; supplemental material available online with this article; doi:10.1172/JCI40331DS1). Consistent with this result, we previously showed that inactivation of p73 in primary mouse embryo fibroblasts (MEFs) impairs proliferation due to activation of p53 (26).

We previously showed that p73 can be a transcriptional target of ectopic Myc in cultured cells (25). In vivo levels of p73 transcripts in premalignant Eμ-myc B cells were mildly higher than those of nontransgenic WT B cells (Figure 2A). This is consistent with the observation that Myc is increased 2- to 4-fold in Eμ-myc B cells (data not shown) and that well-studied transcriptional targets of Myc are slightly increased in B cells of these mice. Overall, p73 might be a direct target of Myc regulation in this cell type. The central mechanism of Myc-induced tumorigenesis is an aberrant expansion of immature B cell population in BM, which accounts for most of the malignant growth of the transformed B cells (22, 23, 27). The absolute number of mature B cells is altered less significantly due to increase in splenic cellularity (Figure 1C). Importantly, forced Myc expression increased the percentage of total B cells in BM of premalignant Eμ-myc mice, independent of the presence or absence of p73 (Supplemental Figure 1A). Furthermore, this B cell hypercellularity was not caused by higher proliferation (Figure 2B) or lower apoptosis (Supplemental Figure 1B), which in fact showed the opposite result in p73-deficient cells, consistent with p53 activation in the absence of p73. The levels of p53 target genes *p21*, *Bax*, and *Noxa* were accordingly increased (Supplemental Figure 1C). However, in contrast to nontransgenic mice, in which p73 plays no apparent role in the maturation of B cells (Figure 2C), the absence of p73 in Myc transgenics shifted the ratio toward maturation. This is indicated by an IgM-negative

Figure 2

In the context of Myc, the absence of p73 allows increased maturation of premalignant B cells. (**A**) Semiquantitative RT-PCR analysis of *p73* mRNA expression in sorted B220+CD19+ B cells isolated from spleens of *p73−/−*, nontransgenic WT and 4 week-old premalignant Eμ-myc mice. HPRT, control for equal cDNA input. (**B**) Proportion of cells in S-phase from total BM of 4-week-old nontransgenic (normal) and premalignant Eμ-myc mice of the indicated genotypes. Error bars represent SEM. (**C**) Cells isolated from spleens of 4-week-old nontransgenic (normal, *n* = 8 per genotype) and premalignant Eμ-myc mice (*n* = 8 per genotype) were stained with antibodies to B220 and IgM and analyzed by FACS. The percentage of B cells is indicated. Error bars represent SEM. (**D**) Cells isolated from BM and spleens of 4-week-old premalignant Eμ-myc mice of the indicated genotypes were stained with antibodies to B220, IgM, and IgD. Proportions of mature B cells (B220+IgM+), recirculating B cells (IgM+IgD+), and T1 transitional and marginal zone B cells (IgMhi/IgDlo) are shown. Error bars represent SEM. (**E**) BM and splenic cells from 3-week-old Eμ-myc mice of the indicated p53 and p73 genotypes were analyzed as in **D**. Error bars represent SEM.

survival of *p73+/−* Eμ-myc mice (*n* = 44) and *p73−/−* Eμ-myc mice (*n* = 30) did not differ significantly when Kaplan-Meier survival curves were analyzed by log-rank test (*P* = 0.127 for WT vs. *p73−/−*; *P* = 0.313 for WT vs. *p73+/−*; overall *P* = 0.291). We conclude that p73 loss has no discernible effect on disease onset and mortality in Myc-induced lymphomagenesis.

p53 but not p73 is the dominant tumor suppressor in Myc-induced lymphomagenesis. We next examined lymphoma onset and latency in genetically matched Eμ-myc *p53+/−*, *p53+/−p73+/−*, and *p53+/−p73−/−* mice. As expected, loss of 1 p53 allele accelerated tumor onset and mortality compared with Eμ-myc *p73−/−* mice (Figure 3B). In contrast, loss of 1 or 2 p73 alleles on the *p53+/−* Myc background had no further impact on survival (Figure 3B). As reported (28, 29), tumors arising in the *p53+/−* Myc background lost the remaining WT p53 allele, consistent with their highly aggressive behavior (Figure 3C), while no loss of heterozygosity (LOH) occurred in the remaining WT p73 allele (Figure 3C). Likewise, single *p73+/−* Myc lymphomas showed no loss of the remaining WT p73 allele (Figure 3D). In sum, these results indicate that p53 but not p73 is the dominant tumor suppressor in Myc-induced lymphomagenesis.

Loss of p73 promotes dissemination of Eμ-myc lymphomas. Histologically, no consistent difference was seen in the morphologic appearance of Eμ-myc *p73+/+* and *p73−/−* lymphomas (Supplemental Figure 2). However, loss of p73 induced a marked alteration in the clinical phenotype of Eμ-myc lymphomas. Thus, *p73+/+* Eμ-myc lymphomas invariably presented as bulky nodal disease with massive splenomegaly and general lymphadenopathy that was easily palpable and dominated the disease picture (representative examples shown in Figure 4, A and B, and Supplemental Figure 3). Eventually, at the advanced stage of disease, tumor dissemination to BM and some other organs occurred (Table 1) (22, 23).

In contrast, a consistent feature of lymphomas arising in *p73−/−* Eμ-myc mice was a lack of the gross degree of splenomegaly and massive lymphadenopathy. Thus, the average nodal tumor burden (spleen weight and combined lymph node weight) of moribund *p73−/−* Eμ-myc was reduced by 33% and 68%, respectively, compared with their *p73+/+* Eμ-myc controls (Figure 4B). Instead of bulky nodal tumors, the disease of tumor-bearing *p73−/−* Eμ-myc mice was marked by systemic signs of illness, including severe apathetic appearance, heavy breathing, and the inability to take food and water. Mice of all genotypes were sacrificed when the strictly

to IgM-positive cell ratio of 5 in WT Eμ-myc mice compared with a ratio of only 1.5 in *p73−/−* Eμ-myc mice, revealing a 3.3-fold increase in the proportion of more mature IgM-positive B cell populations (Figure 2, C and D). Heterozygous *p73+/−* Eμ-myc mice showed an intermediate phenotype. Most of the excess cells in spleens of *p73−/−* Eμ-myc mice were B220+IgM+, IgMhiIgDlo, and IgM+IgD+ lymphocytes comprising T1 transitional, marginal zone, and memory B cell subsets (Figure 2D).

In contrast to nontransgenic p73-deficient mice, analysis of premalignant compound Eμ-myc mice heterozygous for p53 (p53-null Eμ-myc animals could not be analyzed because they die in utero) showed that the differentiation defect associated with p73 deficiency was not significantly rescued by loss of 1 p53 allele and thus is possibly p53-independent (Figure 2E). Based on these aggregate results, we conclude that p73 loss affects B cell numbers by altering their development and/or peripheral differentiation. Moreover, in the context of Myc, the absence of p73 allows increased B cell maturation in premalignant mice.

p73 loss has no discernible effect on disease onset and mortality in Myc-induced lymphomagenesis. WT Eμ-myc mice develop fully penetrant, lethal B cell lymphomas starting at 2 months of age (22, 23). Most WT Eμ-myc mice in our cohort also succumbed to lymphomas within 2–4 months after birth (Figure 3A, *n* = 36). Of note,
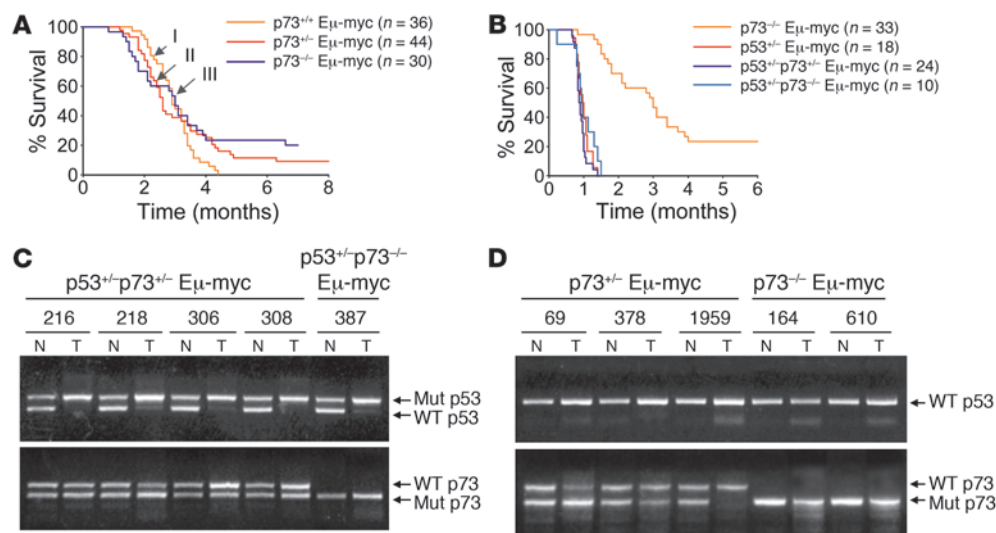
Figure 3

p73 deficiency has no discernible effect on disease onset and mortality in Myc-driven lymphomagenesis. (**A**) Kaplan-Meier survival curves of WT Eμ-myc (curve I), $p73^{+/-}$ Eμ-myc (curve II), and $p73^{-/-}$ Eμ-myc (curve III) mice. The number of animals per genotype is indicated. (**B**) Kaplan-Meier survival curves of Eμ-myc mice of the indicated p53 and p73 genotypes. (**C** and **D**) LOH analysis at the p53 and p73 loci by genomic PCR of matched pairs of normal (N) and tumor (T) tissues derived from Eμ-myc $p53^{+/-}p73^{+/-}$ and Eμ-myc $p53^{+/-}p73^{-/-}$ mice (**C**) or from Eμ-myc $p73^{+/-}$ and Eμ-myc $p73^{-/-}$ mice (**D**). Individual animals are indicated.

defined experimental end point was reached (see Methods). Histological analysis showed that the clinical disease of $p73^{-/-}$ Eμ-myc mice was mainly due to widespread tumor dissemination, the extent of which was considerably higher compared with that of $p73^{+/+}$ Eμ-myc animals (examples in Supplemental Figure 4). Conversely, when tissues from Eμ-myc $p73^{+/+}$ and $p73^{-/-}$ mice were compared whose lymphadenopathy was equal in extent, control tumors were not disseminated but limited to spleens and lymph nodes (data not shown). One third of $p73^{+/-}$ and $p73^{-/-}$ Eμ-myc mice showed dissemination to the lung (Table 1 and Figure 4C), an organ not affected in our control cohort, regardless of tumor burden or leukemic status. Furthermore, while meningeal involvement in control mice was mild, affecting the leptomeninges in 30% of preterminal animals, it was extensive, with massive thickening of the dura mater and leptomeninges in 70% of $p73^{-/-}$ Eμ-myc mice (Table 1). Likewise, while spread to brain parenchyma occurred in 33% of control mice, 83% of $p73^{-/-}$ Eμ-myc mice were affected (Table 1 and Figure 4C). One of the most striking dissemination patterns found in all $p73^{-/-}$ Eμ-myc mice was the presence of macroscopically apparent lymphomatous tumor masses ("ridges") running symmetrically along the vertebral column (Table 1 and Supplemental Figure 4). Histologically, these tumor masses had completely effaced paravertebral skeletal muscles and soft tissues. In contrast, only 10% of control mice were affected (Table 1). Interestingly, the incidence of leukemia was similar between the genotypes (Table 1), suggesting that the widespread tumor dis-

semination into extranodal and extralymphoid tissues upon p73 loss was not simply due to leukemic conversion.

The enhanced dissemination propensity of $p73^{-/-}$ compared with $p73^{+/+}$ lymphoma cells was also reproducible in nude mouse assays. After tail vein injection, $p73^{-/-}$ Eμ-myc lymphoma cells showed greater degree of extranodal growth compared with WT Eμ-myc controls (Figure 4D). In mice receiving $p73^{-/-}$ tumor cells, the entire viscera in the abdominal cavity were completely overrun by tumor masses. Dissemination also extended into abdominal muscles, the paravertebral regions, lymph nodes, brain, lung, and skin. Collectively, these data demonstrate that $p73^{-/-}$ Eμ-myc lymphomas exhibit a greater propensity for widespread dissemination and extranodal growth than WT Eμ-myc control tumors, which is the single major cause of death in tumor-bearing $p73^{-/-}$ Eμ-myc mice.

As already seen with premalignant B cells (Figure 2, C and D), p73 loss also allowed increased maturation in malignant B cells. Thus, 90% of p73-null lymphomas ($n = 15$) expressed surface IgM, indicating a more mature B cell phenotype, compared with only 50% of $p73^{+/+}$ lymphomas ($n = 14$) (Figure 4E; $P = 0.050$, 2-tailed Fisher's exact test). In support, $p73^{-/-}$ lymphomas tended to express increased levels of activation-induced cytidine deaminase (AID) (Figure 4F; 7 randomly chosen lymphomas per genotype), an enzyme normally required in mature B cells for antibody diversification via class switch recombination and somatic hypermutation. When constitutively expressed, AID is involved in germinal center–derived mature B lymphomagenesis by generating DNA double-strand breaks in off-target genes (30, 31). Taken together, while half of our WT Eμ-myc lymphomas were immature lymphoblastic pre–B lymphomas

Table 1

Lymphoma dissemination in Eμ-myc mice with 2, 1, or 0 p73 alleles

Genotype	$p73^{+/+}$	$p73^{+/-}$	$p73^{-/-}$
	$n = 15$	$n = 10$	$n = 15$
Thymus[A]	75%	75%	80%
Liver	90% mild	90% mild	90% mild
Intestine	70% minimal	50% minimal	60% minimal
Kidney	25% minimal	10% minimal	15% minimal
Leukemia	40%	50%	40%
Lung	0%	33%	33%
Meninges	30% lepto, mild	33%	70% dura and lepto, extensive
Brain parenchyma	33%	33%	83% multifocal
Paravertebral "ridges"	10% absent or mild	60% mild to severe	100% moderate to severe

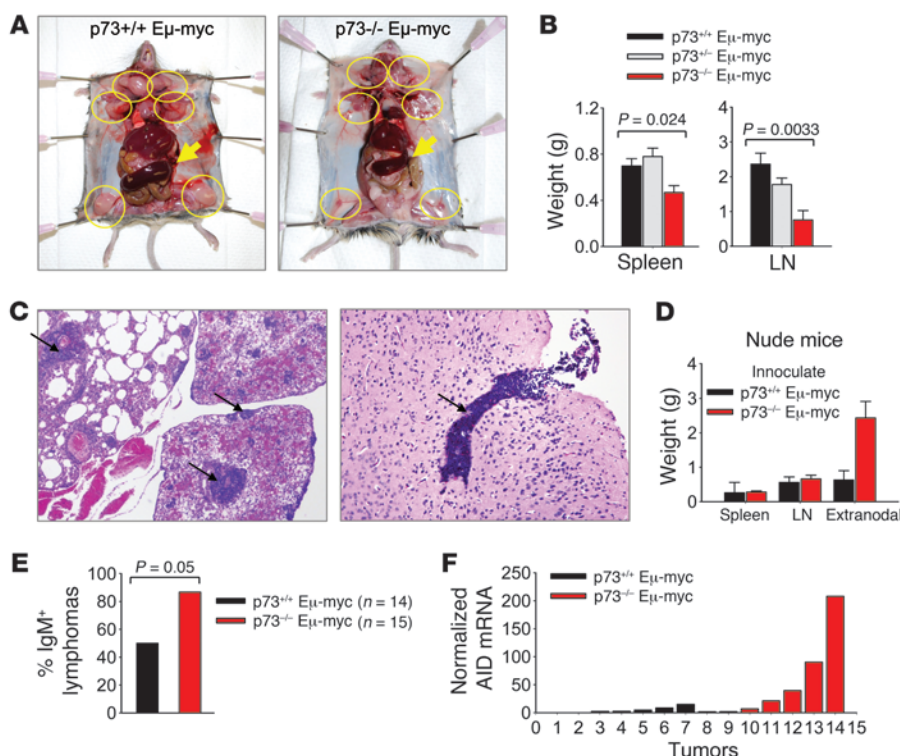[A]Equal range from minimal to severe in all genotypes.

Figure 4
Loss of p73 promotes dissemination of Eμ-myc lymphomas. (**A**) Representative moribund $p73^{+/+}$Eμ-myc and $p73^{-/-}$ Eμ-myc mice are shown. Arrows, spleens; circles, lymph nodes. For comparison, spleens have been placed on top of the bowel. (**B**) Average weight of spleen and lymph nodes (compound per animal) from moribund Eμ-myc $p73^{+/+}$, $p73^{+/-}$, and $p73^{-/-}$ mice. Error bars represent SEM derived from 20 mice of each genotype. (**C**) Histological examination (H&E staining) of lymphoma dissemination in $p73^{-/-}$ Eμ-myc mice. Arrows denote lymphomatous infiltrates in lung and brain parenchyma. Original magnification, ×4 (left); ×10 (right). (**D**) Weight of reconstituted lymphomas in nude mice inoculated with Eμ-myc $p73^{+/+}$ and $p73^{-/-}$ lymphoma cells (1×10^6 cells each). Error bars represent SEM from 4 independent experiments. (**E**) Immunophenotyping of tumors from $p73^{+/+}$ and $p73^{-/-}$ Eμ-myc mice with B220, CD19, and IgM antibodies. Tumors were classified as either predominantly (>80%) IgM-positive (mature) or IgM-negative (immature). (**F**) Quantitative real-time RT-PCR analysis of AID in Eμ-myc $p73^{+/+}$ and $p73^{-/-}$ lymphomas (7 random tumors per genotype).

(23), $p73^{-/-}$ Eμ-myc tumors were predominantly mature aggressive tumors. As such, they display a higher incidence of features resembling diffuse large B cell lymphoma (DLBCL), an important and diverse group of mature aggressive human B cell neoplasms with a high propensity for extranodal dissemination (32).

p73 loss does not confer a proliferative or survival advantage to tumor cells. Impaired apoptosis is central to tumor development. However, contrary to expectations, p73 loss did not confer a growth or survival advantage to the cell. Premalignant $p73^{-/-}$ Eμ-myc B cells from BM did not show an increase in proliferation (Figure 2B) or a decrease in apoptosis (Supplemental Figure 1B). Likewise, malignant $p73^{+/+}$ and $p73^{-/-}$ lymphomas did not differ in their proliferative rates (Figure 5, A and B, and data not shown) and spontaneous apoptosis rates (Figure 5C). Furthermore, $p73^{-/-}$ Eμ-myc tumor cells did not grow well in vitro and rapidly died upon exposure to genotoxic drugs, similar to $p73^{+/+}$ tumor cells (Figure 5D). In contrast, tumors derived from $p53^{+/-}p73^{-/-}$ Eμ-myc mice were refractory to apoptosis (Figure 5D). Thus, although p73 is thought to be one of the downstream mediators of Myc-induced apoptosis in solid cancers (4, 5, 11, 25), p73 loss in Eμ-myc B cells in vivo does not confer an apoptosis defect. This situation is reminiscent of MEFs, in which c-Myc sensitization of etoposide-induced apoptosis is similar between WT and $p73^{-/-}$ MEFs (8). Of possible interest in this context, c-Myc was reported to physically bind to p73, thereby impairing p73's transcriptional activity on the Bax and p21 promoters (33).

In addition to changes in the clinical course and immunophenotype, loss of p73 influenced tumor cytogenetics. Lymphomas from Eμ-myc $p73^{+/+}$ and $p73^{+/-}$ mice were characteristically aneuploid. In contrast, most $p73^{-/-}$ Eμ-myc lymphomas did not display aneuploidy but remained pseudodiploid, as determined by flow cytometry and spectral karyotyping (Supplemental Figure 5 and data not shown).

Because lymphomas arising in WT Eμ-myc mice frequently mutate the p19ARF/p53 pathway (29, 34), we next evaluated p53 and ARF status in tumors arising in $p73^{-/-}$ Eμ-myc mice. Interestingly, while the expected proportion of WT Eμ-myc lymphomas either sustained inactivating p53 mutations (e.g., tumors 74 and 136, sequence confirmed), Mdm2 overexpression (e.g., tumor 140), or other unknown mutations impinging on the pathway (e.g., tumor 13) that collectively are all reflected by aberrant ARF upregulation, $p73^{-/-}$ Eμ-myc lymphomas did not exhibit high levels of p53 protein indicative of p53 mutations (Figure 5E). Sequencing full-length p53 cDNAs from 12 p73-null lymphomas (including tumors shown in Figure 5E) confirmed their WT p53 status. Although WT Eμ-Myc B cells express very low levels of ARF protein as long as disease is latent (35), mutations of p53 disrupt the p53-ARF negative-feedback loop, resulting in strong induction of ARF levels (24). Conversely, the p53 pathway can be crippled by a complete loss of ARF expression (24), even though ARF inactivation is itself insufficient to trigger lymphomagenesis in Eμ-myc mice (35). Of note, while ARF was markedly upregulated in 4 of 10 $p73^{+/+}$ lymphomas, ARF levels remained low in all $p73^{-/-}$ lymphomas (Figure 5E). Relative quantitation by real-time PCR in individual tumors confirmed that *Arf* mRNA levels in WT Eμ-myc lymphomas varied widely, including greatly increased levels in 2 cases and undetectable levels in 4 cases. In contrast, *Arf* mRNA levels displayed little or no change in tumors of $p73^{-/-}$ Eμ-myc mice compared with their premalignant $p73^{-/-}$ Eμ-myc controls or WT Eμ-myc B cells (Figure 5F), confirming WT p53 status of $p73^{-/-}$ tumors. Thus, we surmise that cryptic mutations other than those causing p53 and/or Arf loss of function accompany the conversion of premalignant $p73^{-/-}$ Eμ-myc B cells to tumor cells.

p73 loss deregulates expression of genes involved in lymphocyte homing and lymphoma dissemination. As a step toward characterizing tran-
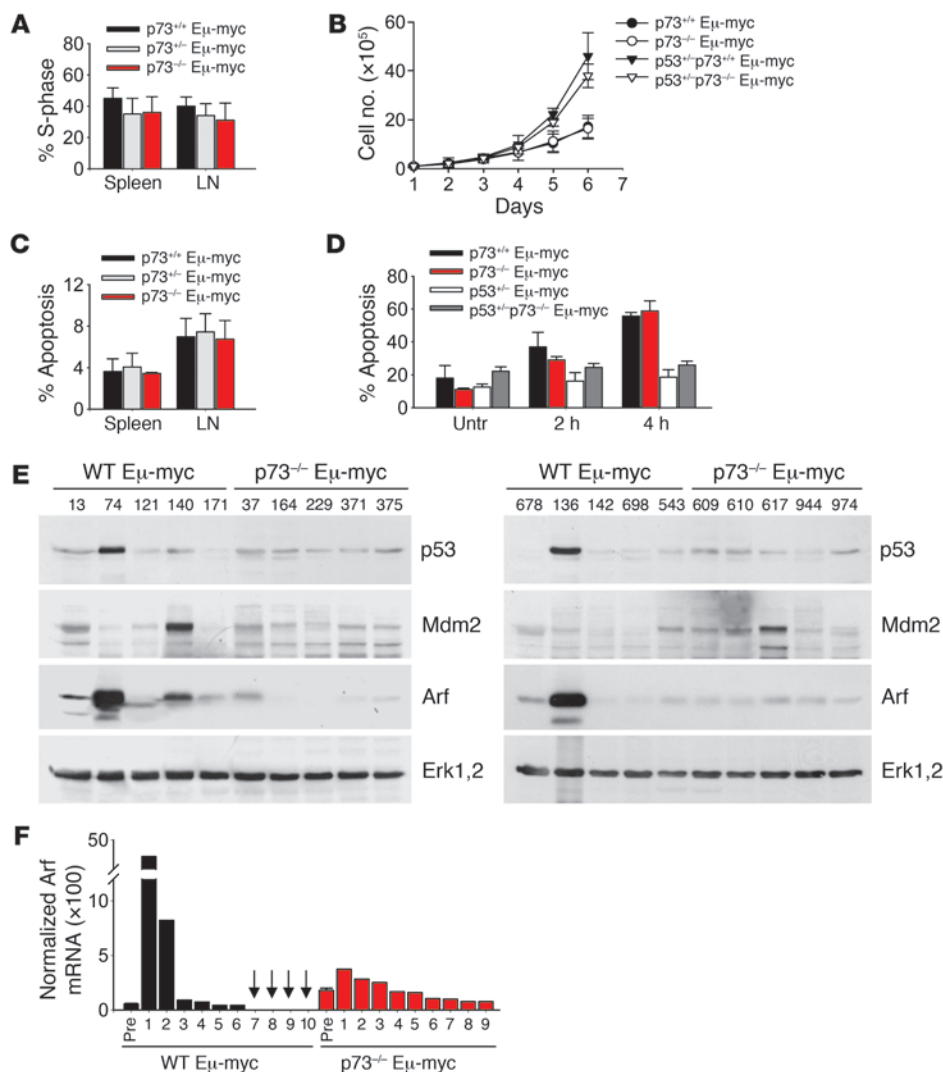
Figure 5
p73 loss does not confer a proliferative or survival advantage to tumor cells. (**A**) S-phase proportion of cells in primary tumors derived from spleens and lymph nodes of Eμ-myc mice of the indicated genotypes (*n* = 10 per genotype). (**B**) In vitro growth curves of Eμ-myc lymphoma cell lines of the indicated genotypes (2 lines each). Identical results were obtained with 2-hour pulse labeling by BrdU. (**C**) Spontaneous apoptosis in primary tumors from spleens and lymph nodes (*n* = 10 per genotype). (**D**) In vitro apoptosis in lymphoma cells (3 cell lines per genotype) that were challenged with 2 μg/ml etoposide for 2 or 4 hours. FACS analysis of propidium iodine–stained (**A**) or annexin V/propidium iodine–stained (**C** and **D**) cells. Error bars represent SEM (**A**–**D**). (**E**) Immunoblot analysis of p53, Mdm2, and Arf expression in Eμ-myc *p73⁺/⁺* and *p73⁻/⁻* lymphomas. Tumor numbers are indicated. Erk1,2, loading control. (**F**) *Arf* mRNA expression, determined by quantitative real-time RT-PCR. Total RNA was isolated from B cells of premalignant (Pre) (*n* = 3 mice per genotype) or tumor-bearing *p73⁺/⁺* (*n* = 10) and *p73⁻/⁻* (*n* = 9) Eμ-myc mice. Numbers denote individual tumors. HPRT levels were used for normalization. Error bars represent SEM.

scriptional profiles that might be relevant in lymphoma dissemination promoted by p73 deficiency, we performed expression analyses of representative tumors from Eμ-myc *p73⁺/⁺* and *p73⁻/⁻* mice. Only tumors with WT p53 were chosen. A total of 822 genes were differentially regulated by at least 2-fold in *p73⁻/⁻* compared with *p73⁺/⁺* lymphomas. Of these, 377 genes were upregulated, while 445 were downregulated in *p73⁻/⁻* tumors. Some of these genes were reported as direct target genes of p73 and/or p53 regulation. Within the upregulated genes, *Timp2* is a p73 target (36), *Ptpre* is a validated novel p53 target, and 5 genes (*Alox5*, *Marco*, *Chn2*, *Abcb9*, and *Gas6*) contain ChIP/PET-verified p53 response elements (37). Within the downregulated genes, *Jag2*, *Trim32*, *Cth*, *Idh2*, *Arntl*, and *Xrcc6* were reported as direct p73 targets (36, 38), *Nck2* is a validated novel p53 target, and 7 genes (*Ank*, *Eno1*, *Pcnx*, *Kcnk1*, *Pmvk*, *Ddit4*, and *Tle1*) contain ChIP/PET-verified p53 response elements (37).

Interestingly, based on Gene Ontology (GO) classification, a large group of up- and downregulated genes (31%, 116 of 377; and 15%, 67 of 445, respectively) in disseminating p73-null lymphomas encoded cell-surface proteins involved in cell adhesion and receptor signaling (Supplemental Table 1; PANTHER functional annotation). For example, p73-null tumors showed upregulation of cell adhesion molecules integrin αL, integrin β7, integrin αX,

and L-selectin. Moreover, p73-null tumors showed upregulation of chemokine receptors (*Ccr2*, *Ccr5*, *Ccr6*, *Cxcr3*, *Cxcr5*, and *Cxcr6*), chemokine ligands (*Cxcl4*, *Ccl5*, and *Ccl6*), and cytokine receptors (*Csf2rb2*, *Csf3r*, *Il18r1*, *Il27ra*, and *Lifr*) (Table 2). Additional deregulated genes in p73-null tumors included hematopoietic stem and progenitor cell–, myeloid lineage–, NK cell lineage–, and T cell lineage–associated genes (Table 2). Of note, adhesion molecules such as L-selectin and integrin β7 as well as chemokine receptors such as CXCR5 are implicated in lymphocyte homing and dissemination of human lymphomas (reviewed in ref. 39). Twenty-three cell-surface genes, shown in Tables 2 and 3, were extensively validated by quantitative real-time RT-PCR in primary malignant lymphomas and premalignant B cells from BM (Figure 6, A and B, and Supplemental Figure 6A). Both premalignant and malignant samples showed consistent p73-dependent changes in the expression profiles of specific genes, indicating that their changes are specific, robust, and occur early during lymphomagenesis (Figure 6, A and B, and Supplemental Figure 6A).

In addition, we asked whether shared deregulated genes exist between disseminating mouse (*p73⁻/⁻* Eμ-myc) and disseminating human B lymphomas. To this end, we queried a published annotated human database of 221 mature aggressive B lymphomas that

Table 2

p73 loss in Eμ-myc lymphoma deregulates expression of genes involved in cell adhesion and receptor signaling that are implicated in lymphocyte homing and human lymphoma dissemination

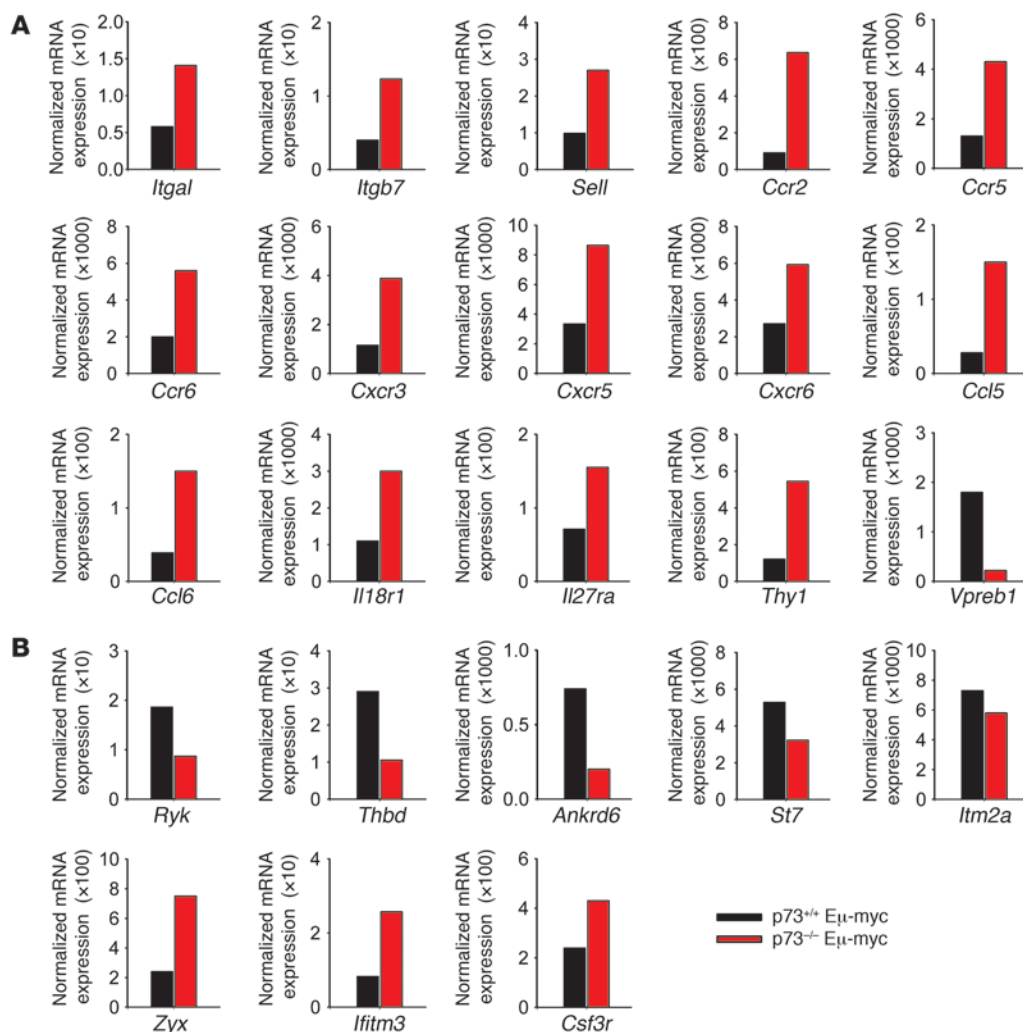| Gene symbol	Gene name	Fold change (log$_2$)	P value
Cell adhesion molecules			
Itgal	Integrin alpha L, CD11A	1.578690254	2.00×10^{-5}
Itgb7	Integrin beta 7	3.361727127	1.08×10^{-8}
Sell	L-selectin	1.73181837	1.82×10^{-6}
Itgax	Integrin alpha X	1.189589482	2.75×10^{-5}
Ifitm3	Interferon-induced transmembrane protein 3	1.827734758	3.85×10^{-5}
Chemokine receptors			
Ccr2	Chemokine (C-C motif) receptor 2	1.106851386	2.05×10^{-5}
Ccr5	Chemokine (C-C motif) receptor 5	1.077494852	3.88×10^{-5}
Ccr6	Chemokine (C-C motif) receptor 6	1.441494716	5.99×10^{-6}
Cxcr3	Chemokine (C-X-C motif) receptor 3	1.159909187	7.83×10^{-5}
Cxcr5	Chemokine (C-X-C motif) receptor 5, Burkitt lymphoma receptor 1 (Blr1)	2.091135729	1.82×10^{-6}
Cxcr6	Chemokine (C-X-C motif) receptor 6	1.08243399	9.19×10^{-5}
Chemokine ligands			
Cxcl4	Chemokine (C-X-C motif) ligand 4	1.32766983	5.35×10^{-5}
Ccl5	Chemokine (C-C motif) ligand 5	2.30735947	2.10×10^{-7}
Ccl6	Chemokine (C-C motif) ligand 6	2.512559223	2.45×10^{-7}
Cytokine receptors			
Csf2rb2	Colony-stimulating factor 2 receptor, beta 2	1.499594383	1.17×10^{-5}
Csf3r	Colony-stimulating factor 3 receptor, granulocyte	1.073218776	1.80×10^{-5}
Il18r1	Interleukin 18 receptor 1	1.032430758	4.71×10^{-5}
Il27ra	Interleukin 27 receptor, alpha	1.500732887	7.49×10^{-6}
Lifr	Leukemia inhibitory factor receptor	1.169730543	4.67×10^{-5}
Hematopoietic stem and progenitor associated			
Cd34	CD34 antigen	1.151022982	1.93×10^{-5}
Cd52	CD52 antigen	2.590008998	1.00×10^{-5}
Myeloid lineage associated			
Cd68	CD68 antigen	1.193065414	1.64×10^{4}
Cd300lf	CD300 antigen-like family member F	1.350391436	1.63×10^{-5}
NK lineage associated			
Cd96	CD96 antigen	1.196468788	2.97×10^{-5}
Cd244	CD244 natural killer cell receptor 2B4	1.933225205	8.14×10^{-7}
T lineage associated			
Cd2	CD2 antigen	1.250522156	5.93×10^{-5}
Cd3g	CD3 antigen, gamma polypeptide	2.574810437	2.23×10^{-7}
Cd3d	CD3 antigen, delta polypeptide	3.167400834	2.40×10^{-7}
Cd5	CD5 antigen	2.555166265	1.39×10^{-7}
Cd6	CD6 antigen, transcript variant 2	1.747161954	1.52×10^{-6}
Cd8b1	CD8 antigen, beta chain 1	4.521213686	3.25×10^{-9}
Cd83	CD83 antigen	1.969812658	3.52×10^{-6}
Thy1	Thymus cell antigen 1, theta	3.87025413	4.46×10^{-9}

had been characterized in great detail (GEO GSE4475) (40). Based on transcriptional and genomic profiling, tumors belonged to 1 of 3 groups (molecular Burkitt lymphoma [mBL]; intermediate group; and non-mBL, the latter largely corresponding to DLBCL and primary mediastinal large B cell lymphoma). Of note, p73 expression was downregulated in 82% of the human mBL lymphomas and in 49% of the intermediate and non-mBL lymphomas (Figure 7A). This confirms and extends previous studies that report loss of p73 expression in nearly one-third of non-Hodgkin B lymphomas (16–19).

We next analyzed in the human lymphoma cohort whether p73 downregulation is relevant to dissemination. Dissemination status was known for 131 of these 221 tumors, of which 65 presented as extranodal (with or without additional nodal disease, grouped as ExN) and 66 presented as nodal (N) lymphomas. A statistically significant correlation between incidence and degree of p73 downregulation and lymphoma dissemination was identified. In extranodal tumors, 66% (43 of 65 cases) exhibited downregulated p73 compared with only 47% (31 of 66 cases) of nodal tumors ($P = 0.0345$) (Figure 7B). Moreover, extranodal lymphomas exhibited lower p73 expression than nodal lymphomas (Figure 7C; all 131 cases; $P = 0.01609$). This correlation was further strengthened within the "low p73" subset of lymphomas (i.e., those with over 2-fold p73 downregulation compared with the mean of the entire 221 case cohort) (Figure 7C; $P = 0.0000502$). The 31 downregulated nodal cases consisted of 7 mBL, 7 intermediate type, and 17 non-mBL. The 43 downregulated extranodal cases consisted of 15 mBL, 10 intermediate type, and 18 non-mBL lymphomas.

Interestingly, among the 445 downregulated mouse genes, 35 genes (8%) are codownregulated in all disseminating mouse and human lymphomas (Table 3 and Supplemental Figure 6B). Among them are 9 cell-surface–expressed genes. These include *Ryk*, a member of the receptor tyrosine kinase family that functions as a receptor for Wnt proteins; thrombomodulin, a cell-surface anticoagulant glycoprotein; and integral membrane protein 2A, which is normally induced during thymocyte selection and T cell activation (Table 3 and Figure 6B).

Furthermore, when each case was analyzed individually, 92% of the extranodal human lymphomas (60 of the 65 cases) shared at least 50% of their downregulated gene list with the downregulated mouse gene list derived from *p73*$^{–/–}$ Eμ-myc mice with disseminating lymphoma. Conversely, 17% of extranodal human lymphomas (11 of 65 cases) shared at least 50% of their upregulated gene list with the upregulated mouse gene list. Among them, 9 genes overlapped, in that they were upregulated in all disseminating mouse and those human lymphomas with more than 50% overlap, i.e., the subset of 17% (Table 3 and Supplemental Figure 6B). (In a random overlap model one would expect 0.198 genes.) Five of these 9 genes were cell-surface genes. They included interferon-induced transmembrane protein 3 (*Ifitm3*), normally expressed on migratory primordial germ cells modulating cell adhesion. *Ifitm3* acts as a homing signal and enables cells to respond to environmental cues that guide their localization (41). Other notable genes are the colony-stimulating factor 3 receptor (*Csf3r*) and zyxin, a cytoskeletal protein involved in cell adhesion (42) (Figure 6B). In

Figure 6
Validation of select genes from microarray analysis by quantitative real-time RT-PCR. (**A** and **B**) Quantitative real-time RT-PCR analysis of genes relevant to tumor dissemination in Eμ-myc mice. In **B**, the selection is based on overlap of deregulated genes in mouse and human B lymphomas. Shown are average expression levels in WT and *p73*$^{-/-}$ Eμ-myc lymphomas (5 pooled tumors per genotype, equal cDNA input per tumor, all tumors harbor WT p53). HPRT used for normalization, samples run in duplicate.
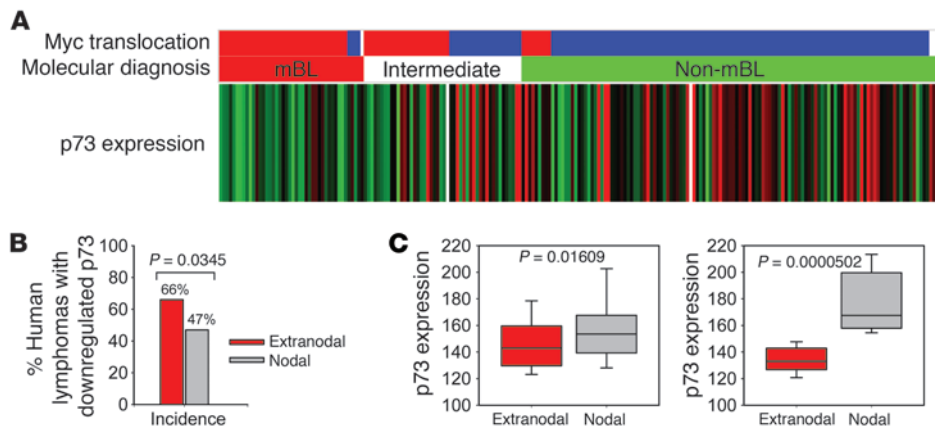
sum, p73 loss associated with mouse lymphoma dissemination alters the transcriptome significantly, including deregulated expression of cell-surface genes known to be involved in lymphocyte homing and dissemination of human lymphomas (39).

Discussion
Here we provide what we believe are novel insights into the role of p73 in B cell lymphomagenesis using the Eμ- myc model. We show that p73 plays an important function in normal B cell development, since its depletion decreases immature and mature B cell populations in primary and secondary lymphoid organs by 30%–40% in a p53-dependent manner. Interestingly, we discovered that the shortened lifespan of nontransgenic *p73*$^{-/-}$ mice can be rescued, enabling us to follow onset and development of B lymphomas after crossing them with Eμ-myc mice. We found that p73 loss has no discernible effect on disease onset and mortality in Myc-driven lymphomagenesis. Instead, the main effect of p73 loss in this context is an altered course of disease associated with a change

in clinical phenotype, whereas effects on cell growth or apoptosis, as one might have expected from p73 studies in cultured cells, are not observed. The phenotype of p73-null Eμ-myc mice compared with WT Eμ-myc controls is characterized by decreased nodal disease in lymphoid organs, but increased propensity to widespread extranodal dissemination. While 50% of WT Eμ-myc lymphomas in our cohort were immature surface IgM-negative pre/pro–B lymphoblastic tumors (23, 43), 90% of p73-null Eμ-myc mice developed more mature surface IgM-positive B cell neoplasms. As such, they displayed a higher incidence of features resembling DLBCL, a major group of mature aggressive human B cell neoplasms with a high propensity for extranodal dissemination (32). Of note, dissemination in human DLBCL confers a poorer prognosis (44).

To delineate functional distinctiveness versus redundancy between p73 and p53, we generated Eμ-myc compound mice deficient in both genes. Our results indicate functional complementarity, in that p53 acts as the dominant tumor suppressor in the onset of B lymphomas, while p73 is a tumor modulator

Figure 7

Correlation between p73 downregulation and dissemination in mature aggressive human lymphomas. (**A**) Expression of the *TP73* gene in 221 human cases of mature aggressive B lymphomas analyzed in ref. 40. Cases with p73 downregulation (green), upregulation (red), or no change (black) compared with the average p73 expression of all 221 cases are indicated. White, not available. The middle bar indicates classification by molecular diagnosis according to ref. 40; molecular Burkitt lymphoma (red); intermediate (white); non-mBL (green). The top bar indicates the presence or absence of Myc translocation; positive (red); negative (blue); not available (white). (**B**) Incidence of human extranodal and nodal lymphomas with downregulated p73. (**C**) Relative levels of *p73* mRNA expression in extranodal (E×N) versus nodal (N) lymphomas. Box plot analyses based on all E×N (*n* = 65) and N (*n* = 66) lymphomas (left), or E×N (*n* = 43) and N (*n* = 31) lymphomas (right) classified as exhibiting greater than 2-fold downregulation of p73 with respect to the entire 221 case cohort. The horizontal line within the boxes indicates the median expression level, with the lower and upper box representing the 25th and 75th percentile, respectively. Whiskers demarcate the 10th and 90th percentile.

(e.g., L-selectin and integrins), chemokine receptors, and chemokine ligands, which are also known to be involved in lymphocyte homing programs (39, 49–51). We show here in a genetically defined mouse model that p73 loss promotes extralymphatic dissemination of Myc-driven lymphoma and alters the transcriptional signature of the malignant B cells, including cell-surface–associated adhesion molecules, chemokine receptors, their ligands, and cluster of differentiation antigens. We find a parallel situation in a well-characterized large cohort of human mature aggressive B lymphomas. In particular, p73 expression was downregulated in 82% of human Burkitt lymphomas and 49% of Burkitt-like and DLBCL lymphomas. Most interestingly, these human lymphomas show a statistically significant correlation between incidence and degree of p73 downregulation and extranodal dissemination status. Moreover, deregulated genes that are shared between disseminating mouse and human B lymphomas can be identified. The prognostic value of p73 inactivation has been established in ALL, where p73 gene methylation was found in 21% of newly diagnosed ALL patients and correlated with unfavorable outcome (19). Unfavorable prognosis upon p73 loss was also confirmed by another independent study that used 251 ALL patients (19). The prognostic value of p73 inactivation in human NHL has been speculated upon, but no clinical outcome data are available yet (reviewed in ref. 19). Our data suggest a hitherto unknown pathogenetic role of p73 as a lymphoma modifier, favoring dissemination. As such, p73 might be a putative biomarker for human B lymphoma dissemination, a notion that can now be tested in clinicopathologic correlation studies.

whose contribution lies mainly in preventing tumor dissemination and extranodal growth. Thus, in this system, p73 functions as a tumor modifier and not as classic tumor suppressor and loss of p73 expression is likely to be a late event in the course of B lymphoma evolution. The fact that p73-null mice are not cancer prone but develop tumors only when crossed into Eμ-myc mice further argues that at least in this oncogenic context, p73 is not a primary tumor suppressor, and its deficiency would likely come into effect when B lymphomas are established. On the other hand, our data raise the possibility that in nascent tumor cells, oncogenic signals render p53 partly dependent on p73 function. In this respect, the absence of p53 mutations in p73-null Eμ-myc tumors is somewhat surprising but not unprecedented. Loss-of-function mutations in Arf, ATM, Bim, or Bax, or overexpression of Bcl2 also cooperate with Myc and partly alleviate the pressure to inactivate p53 during lymphomagenesis (34, 45–48). As such, it will be important to delineate how specific oncogenic factors might influence the functional interactions between p53 and p73.

Human lymphoid malignancies are predominantly B cell in origin. They mainly belong to the NHL group, of which Burkitt lymphoma and DLBCL are important subgroups, and often involve chromosomal translocations among protooncogenes such as c-Myc, Bcl1, Bcl2, and Bcl6 and the immunoglobulin or other active loci (20). The proliferation and dissemination are equally important for lymphoma prognosis (39). Thus, in the International Prognostic Index for aggressive NHL, the number of extranodal sites is one of 4 prognostic variables (44). However, the molecular biology of lymphoma dissemination is incompletely understood and so far mainly based on clinical observations. Up until now, an important role in dissemination has been established for cell adhesion molecules

Methods

Mice. All animal studies were approved by the Institutional Animal Care and Use Committee at Stony Brook University. The study was littermate controlled and performed in a defined way (see below). Trp73-knockout mice (gift from F. Mckeon, Harvard Medical School, Boston, Massachusetts, USA; ref. 12) were maintained on the 129Sv background. *p73*^{+/–} mice were interbred with C57BL/6 Eμ-myc mice (Jackson Laboratory) to obtain the F1 generation of Eμ-myc *p73*^{+/–} transgenic animals. These were interbred with *p73*^{+/–} mice to obtain the F2 generation Eμ-myc *p73*^{+/+}, *p73*^{+/–}, and *p73*^{–/–} transgenic animals (129 × B6; 75:25). *p53*^{+/–}*p73*^{+/–} double-heterozygous mice were derived by crossing *p73*^{+/–} with *p53*^{–/–} mice (Trp53tm1Tyj on the 129Sv background, Jackson Laboratory). *p53*^{–/–}*p73*^{+/–} mice were derived from crosses between the double-heterozygous animals. *p53*^{–/–}*p73*^{–/–} mice were derived from crosses between *p53*^{+/–}*p73*^{+/–} mice or *p53*^{+/–}*p73*^{+/–} with *p53*^{–/–}*p73*^{+/–} mice. *p73*^{+/–} Eμ-myc transgenic mice (129 × B6) were crossed with *p53*^{–/–}*p73*^{+/–} mice (129Sv) to obtain the F1 generations of Eμ-myc *p53*^{+/–}, *p53*^{+/–}*p73*^{+/–}, and *p53*^{+/–}*p73*^{–/–} transgenic animals (129 × B6; 75:25). Genotypes were verified by PCR amplifications specific for the corresponding WT and mutant alleles. All

Table 3
Shared up- and downregulated genes in mouse and human lymphomas

Gene symbol	Gene name
Shared downregulated genes in disseminated mouse lymphomas	
Cell surface	
Ryk	Receptor-like tyrosine kinase
Thbd	Thrombomodulin
Ankrd6	Ankyrin repeat domain 6
St7	Suppression of tumorigenicity 7
Itm2a	Integral membrane protein 2A
Ssfa2	Sperm-specific antigen 2
Col18a1	Procollagen, type XVIII, alpha 1
St3gal5	ST3 beta-galactoside alpha-2,3-sialyltransferase 5
Slamf7	SLAM family member 7
Other	
Anxa9	Annexin A9
Mtmr3	Myotubularin related protein 3
Gfi1	Growth factor independent 1
Aco1	Aconitase 1
Asah1	N-acylsphingosine amidohydrolase 1
Gna12	Guanine nucleotide binding protein, alpha 12
Rragd	Ras-related GTP binding D
Arhgap24	Rho GTPase activating protein 24
Slc39a6	Solute carrier family 39 (metal ion transporter), member 6
Dusp22	Dual specificity phosphatase 22
Ptpla	Protein tyrosine phosphatase-like (proline instead of catalytic arginine), member
Acsl1	Acyl-CoA synthetase long-chain family member 1
Farp1	FERM, RhoGEF (Arhgef) and pleckstrin domain protein 1
Farp2	FERM, RhoGEF and pleckstrin domain protein
Arntl	Aryl hydrocarbon receptor nuclear translocator-like
Acsl4	Acyl-CoA synthetase long-chain family member 4, transcript variant 1
Pcnx	Pecanex homolog (Drosophila)
Aph1b	Anterior pharynx defective 1b homolog (Caenorhabditis elegans)
Tle1	Transducin-like enhancer of split 1, homolog of Drosophila E (spl)
Mgll	Monoglyceride lipase
Freq	Frequenin homolog (Drosophila)
Plk2	Polo-like kinase 2
Rnf125	Ring finger protein 125
Atp1b1	ATPase, Na$^+$/K$^+$ transporting, beta 1 polypeptide
Gadd45g	Growth arrest and DNA-damage-inducible 45 gamma
Tmod1	Tropomodulin 1
Shared upregulated genes in disseminated mouse and human lymphomas	
Cell Surface	
Zyx	Zyxin
Ifitm3	Interferon-induced transmembrane protein 3
Csf3r	Colony-stimulating factor 3 receptor (granulocyte)
P2ry6	Pyrimidinergic receptor P2Y, G-protein coupled, 6
Cybb	Cytochrome b–245, beta polypeptide
Other	
S100a11	S100 calcium-binding protein A11 (calgizzarin)
Cd63	Cd63 antigen
Ifi30	Interferon gamma inducible protein 30
Hk3	Hexokinase 3

animals were genotyped twice, at the time of weaning and when sacrificed. Our experimental end point was defined in the following way: mice were monitored every day for palpable tumors. The maximum tumor size allowed did not exceed 1 cm³. Mice were also monitored for systemic signs of illness, apathy, breathing problems, precipitous weight loss, and limited ability to reach food or water. This was particularly obvious in *p73⁻/⁻* Eµ-myc because they predominantly developed systemic disease rather than palpable tumors. Mice fulfilling any of these criteria were euthanized and subjected to careful necropsy examination. Tumors were harvested and processed for histological examination. For in vivo dissemination assays, 1 million freshly isolated lymphoma cells was injected into the tail veins of CD-1 athymic nude mice (Taconic Farms). After 25 days, reconstituted mice were subject to autopsy; tumors were weighed and tissues processed histologically.

Flow cytometry. Fresh lymphoid tissue samples were examined by flow cytometry with antibodies to CD19, CD25, CD43, CD44, B220 (CD45R), IgM, IgD, c-Kit, and Sca1 (BD Pharmingen and eBioscience). For cell-cycle analysis, lymphoid cells were fixed in 70% ethanol, stained with propidium iodide, and analyzed using FACSCalibur (BD) with CellQuest software. Annexin V (Roche) staining was performed to measure apoptosis.

Expression analysis. Semiquantitative and quantitative real-time RT-PCR were performed using whole-cell RNAs prepared from primary lymphoid cells or tumors. The sequences of primers are listed in Supplemental Table 2. For protein analysis, aliquots of whole-cell lysates (50–80 µg of protein) were separated on SDS-acrylamide gels and blotted onto a Protran BA85 nitrocellulose membrane (Schleicher & Schüell). They were then incubated with antibodies specific for p53 (FL-393), Mdm2 (SMP14, both from Santa Cruz Biotechnology Inc.); p19ARF (ab80, Novus Biologicals); and MAPK (3A7; Cell Signaling). Comparative genomic hybridization (CGH analysis) and Spectral karyotyping (SKY) were performed at the Roswell Park Cancer Institute (Buffalo, New York, USA).

Expression microarray. Only tumors that showed at least 95% B cell enrichment by FACS analysis were selected. Total RNA was extracted from representative tumors of 3-month-old Eµ-myc *p73⁺/⁺* and Eµ-myc *p73⁻/⁻* mice (3 animals per genotype) using the RNeasy Mini Kit (QIAGEN). The integrity of RNA was validated with Bioanalyzer 2100 QC (all samples RIN > 7.0). One-color hybridizations of labeled cRNAs (2 technical replicas) were performed against the mouse Agilent 44K/60-mer oligonucleotide microarray according to the manufacturer's instructions (Empire Genomics LLC). Normalized, differentially expressed genes were defined as greater than 2-fold change and $P < 0.05$ (Student's *t* test).

Statistics. Statistical analyses were performed using the following tests: a single-factor ANOVA at a 95% level of confidence (Figure 2B and Figure 4B); the log-rank test (Figure 3A); Fisher's exact test, 2-tailed (Figure 4E and Figure 7B); and Student's *t* test, 2-tailed (Figure 7C). $P \leq 0.05$ was considered statistically significant.

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