RESEARCH COMMUNICATION

Dmp1 is haplo-insufficient for tumor suppression and modifies the frequencies of *Arf* and *p53* mutations in *Myc*-induced lymphomas

Kazushi Inoue,[1] Frederique Zindy,[1] David H. Randle,[1,4] Jerold E. Rehg,[2] and Charles J. Sherr[1,3–5]

Department of [1]Tumor Cell Biology, [2]Pathology, and [3]Howard Hughes Medical Institute, St. Jude Children's Research Hospital, Memphis, Tennessee 38105, USA; [4]Department of Molecular Sciences, University of Tennessee College of Medicine, Memphis, Tennessee 38163, USA

Loss of Dmp1, an *Arf* transcriptional activator, leads to spontaneous tumorigenesis in mice, causing death from various forms of cancer by two years of age. Retention and expression of the wild-type *Dmp1* allele in tumors arising in *Dmp1*$^{+/-}$ mice demonstrate that *Dmp1* can be haplo-insufficient for tumor suppression. The mean latency of Eμ-*Myc*-induced B-cell lymphomas is halved on a *Dmp1*$^{-/-}$ or *Dmp1*$^{+/-}$ genetic background. Although *p53* mutations or *Arf* deletion normally occur in ~50% of Eμ-*Myc*-induced lymphomas, *Dmp1* loss obviates selection for such mutations, indicating that *Dmp1* is a potent genetic modifier of the Arf–p53 pathway in vivo.

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Induction of p53-dependent transcription after genotoxic stress or oncogene activation leads to expression of genes that trigger either cell cycle arrest or apoptosis, thereby guarding against tumor formation (Levine 1997). The central role of p53 in tumor suppression is underscored by the fact that about half of human cancers have *p53* mutations, whereas many of the remainder exhibit particular alterations in other genes, such as *Mdm2* and *Arf*, which disable p53 function.

The best characterized negative regulator of p53 is Mdm2, which ubiquitinates p53 and targets its proteolytic destruction (Prives 1998; Juven-Gershon and Oren 1999). Because *Mdm2* is itself a p53-inducible gene, it participates in a negative feedback loop that helps to terminate the p53 response. In turn, by antagonizing functions of Mdm2, the product of the *Arf* tumor suppressor gene (p19^{Arf} in mouse and p14^{ARF} in humans) stabilizes p53 and enhances its activity (Sherr 1998; Sharpless and DePinho 1999). *Arf* is induced by oncogenes such as *Myc*, *E1A*, *Ras*, and v-*Abl*, and also by overexpression of E2F transcription factors whose normal role is to control the temporal expression of genes required for DNA replication. By monitoring the strength of mitogenic signals, *Arf* prevents hyperproliferation by diverting incipient cancer cells to alternative fates—namely, p53-dependent growth arrest, or more dramatically, apoptosis.

Arf disruption in mice predisposes to spontaneous tumor development (Kamijo et al. 1997) and enhances the rate at which mouse strains sustaining other oncogenic lesions develop tumors (Chin et al. 1997; Holland et al. 1998; Eischen et al. 1999; Jacobs et al. 1999b; Schmitt et al. 1999). Pertinent to studies described below, more than half of the Burkitt-type B-cell lymphomas arising in Eμ-*Myc* transgenic mice were found to sustain *p53* mutations (28%) or biallelic *Arf* deletions (24%), whereas others lacking overt *Arf* or *p53* mutations overexpressed Mdm2 (16%) (Eischen et al. 1999). The 25-wk mean latency for tumor formation in Eμ-*Myc* transgenic mice was halved on the *Arf*$^{+/-}$ background, and 80% of the resulting lymphomas lost the wild-type *Arf* allele. *Arf*-null, Eμ-*Myc* transgenic animals developed more aggressive lympholeukemias and died by only 8 wk of age. Together, these results emphasize the role of the Arf–Mdm2–p53 pathway in protecting against tumor emergence. However, we also observed that some Eμ-*Myc* lymphomas that had deleted the *Arf* gene or sustained *p53* mutations overexpressed Mdm2, implying that the Arf–Mdm2–p53 pathway is not strictly linear. Moreover, about a quarter of the lymphomas arising in Eμ-*Myc* transgenic mice exhibited no identifying genetic lesion in *Arf*, *Mdm2*, or *p53*, suggesting that other mechanisms may deregulate the pathway.

A series of transcription factors other than E2F and Myc govern *Arf* expression. These include other positively acting factors, such as Dmp1 (see below) and β-catenin/Tcf (M. Oren, pers. comm.) that bind directly to the *Arf* promoter to induce transcription, as well as proteins such as Bmi-1 (Jacobs et al. 1999a), Tbx-2 (Jacobs et al. 2000), Twist (Maestro et al. 1999), Jun D (Weitzman et al. 2000), and p53 itself (Kamijo et al. 1998; Stott et al. 1998), which negatively regulate *Arf* through as yet ill-defined mechanisms. Overexpression of Twist and Tbx-2 in human rhabdomyosarcomas and breast carcinomas, respectively, suggests that active *ARF* repression might contribute to tumorigenesis. Epigenetic silencing of the *ARF* promoter has also been observed in human cancers (Esteller et al. 2001).

Dmp1 was isolated in a two-hybrid interactive screen using cyclin D2 as bait, and it encodes a 120- to 130-kD nuclear phosphoprotein with a central DNA-binding domain containing three Myb-like repeats flanked by acidic transactivation domains at both termini (Hirai and Sherr 1996; Inoue and Sherr 1998). The human and murine orthologs share 95% amino-acid sequence identity and are completely conserved throughout their Myb-like repeats (Bodner et al. 1999). Dmp1 binds to nonameric Ets consensus sequences in DNA (CCCG[G or T]ATGT) and competes with Ets-family proteins for sites that contain the GGA core. DNA binding can be antagonized by the interaction of Dmp1 with D-type, but not other cyclins. Despite the frequency of potential Dmp1-binding sites in mammalian genomes, its binding

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[5]Corresponding author.
E-MAIL sherr@stjude.org; FAX (901) 495-2381.
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to a single site in the proximal *Arf* promoter induces $p19^{Arf}$-dependent cell cycle arrest (Inoue et al. 1999). Conversely, *Arf* expression is dampened in *Dmp1*-null mouse embryo fibroblasts (MEFs), which resist cellular senescence, do not rapidly accumulate *p53* mutations, and are susceptible to transformation by oncogenic *Ras* (Inoue et al. 2000). *Dmp1*-null mice did not spontaneously develop cancers in their first year of life but were susceptible to carcinogen- and radiation-induced tumors, implying that by regulating *Arf* function, Dmp1 may have tumor-suppressing activities (Inoue et al. 2000). Here, we show that *Dmp1* is a bona fide tumor suppressor gene whose loss obviates the selection for *Arf* deletion or *p53* mutation that normally occurs during *Myc*-induced lymphomagenesis.

Results and Discussion

Both $Dmp1^{-/-}$ *and* $Dmp1^{+/-}$ *mice spontaneously develop tumors*

Dmp1-null mice spontaneously developed lethal tumors in their second year of life with a mean latency of 83 wk (Fig. 1). The most frequently encountered tumors were pulmonary adenomas (27% incidence) and adenocarcinomas (15%). Hepatocellular tumors (18%), B-cell lymphomas (15%), and vascular tumors, including hemangiomas (15%) and hemangiosarcomas (9%) were also relatively common. (For a detailed listing of tumor types for all cohorts, go to http://www.genesdev.org for Supplemental Materials, part A; for representative tumor histology, see Supplemental Materials, part B.) The time of appearance and variety of tumors observed in *Dmp1*-null animals bear no obvious relationship to those in *Arf*-null animals, which exhibit a different spectrum (43% sarcomas, 29% lymphomas, 17% carcinomas, 11% central nervous system tumors) and a shorter mean latency (38 wk) (Kamijo et al. 1999).

Treating *Dmp1*-null mice neonatally with dimethylbenzanthracene (DMBA) or ionizing radiation accelerated tumorigenesis (Fig. 1), and many such animals developed multiple tumors of more than one histological type (Inoue et al. 2000). Lung tumors (46% adenomas and 26% adenocarcinomas) and epidermal tumors of skin (36%) predominated in DMBA-treated *Dmp1*-null mice.

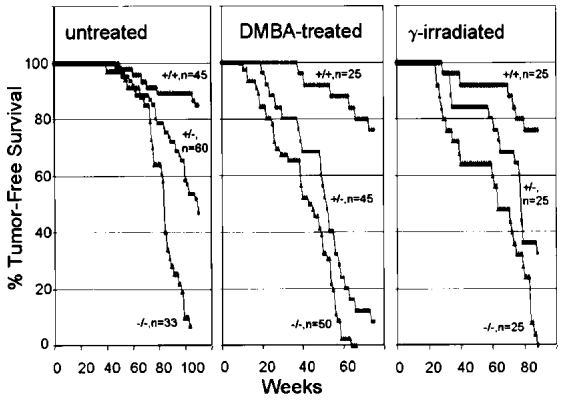

Figure 1. Tumor-free survival in cohorts of untreated $Dmp1^{+/+}$, $Dmp1^{+/-}$, and $Dmp1^{-/-}$ mice (*left*) and in animals neonatally exposed to DMBA (*middle*) or X rays (*right*). The numbers of animals in each group is indicated by the corresponding survival curve.

Many developed ovarian tumors mostly of granulosa cell origin (40% of females), lymphomas (14%), and melanomas (12%). Irradiated *Dmp1*-null mice were prone to lymphoid malignancies (32%) predominantly of T-cell origin (24%), ovarian tumors (56% of females), lung and hepatocellular tumors (each 12%).

Interestingly, $Dmp1^{+/-}$ heterozygotes were also tumor prone (Fig. 1). A broad spectrum of tumors was seen in each of these cohorts, although the frequencies of tumor types were somewhat different from those in *Dmp1*-null mice (see Supplemental Materials, part A). Given the number of animals in each group, the latter differences may not be significant. However, the rates of tumor appearance in $Dmp1^{+/-}$ animals clearly eclipsed those of $Dmp1^{+/+}$ littermate controls. These observations raised the question of whether the wild-type *Dmp1* allele was lost or epigenetically suppressed, or whether *Dmp1* might be haplo-insufficient for tumor suppression. The wild-type *Dmp1* locus was retained in four of four T-cell lymphoblastic lymphomas from DMBA-treated $Dmp1^{+/-}$ mice, and Dmp1 protein was detected by immunoblotting (Fig. 2A). Lung adenocarcinoma samples from untreated and DMBA-treated $Dmp1^{+/-}$ mice also retained the wild-type allele and expressed *Dmp1* mRNA at levels that approximated those in normal lung tissue (Fig. 2B). The relatively small differences in mean latencies of appearance of T-cell lymphomas (25 and 28 wk, respectively, for $Dmp1^{-/-}$ and $Dmp1^{+/-}$ genotypes) and lung tumors (50 and 60 wk, respectively) in DMBA-treated mice are consistent with the idea that *Dmp1* is haploinsufficient for suppression of both tumor types (see below). In two lung tumors (K938 and K2130), a reduced Southern blot signal for the wild-type *Dmp1* allele correlated with a reduction in *Dmp1* mRNA expression, suggesting that the normal allele was lost from some cells. Our data do not preclude such events, but argue that segregation of the wild-type allele is relatively uncommon in these tumor types.

Because expression of the other product of the *Ink4a/Arf* locus ($p16^{Ink4a}$) can be epigenetically silenced in lung tumors (Merlo et al. 1995; Swafford et al. 1997; Herzog et al. 1999; Patel et al. 2000), we used methylation-specific PCR assays (Herman et al. 1996) to survey the status of the *Ink4a* and *Arf* promoters (Randle et al. 2001). Importantly, lung tumors retained the *Ink4a/Arf* locus. *Ink4a* promoter methylation was detected in four of eight such samples, whereas *Arf* was unaffected (see Supplemental Materials, part C). Therefore, *Ink4a* (p16) silencing likely contributes to pulmonary carcinogenesis in the $Dmp1^{+/-}$ background, whereas there is no apparent selection for *Arf* loss.

Dmp1 *loss accelerates Eμ-*Myc*-induced lymphomagenesis without frequent* p53 *mutation or* Arf *deletion*

Eμ-*Myc* transgenic mice develop Burkitt-type B-cell tumors (Adams et al. 1985) with a mean latency of about 6 mo in our strains (Eischen et al. 1999). When crossed onto a $Dmp1^{+/-}$ or $Dmp1^{-/-}$ background, lymphomagenesis induced by the Eμ-*Myc* transgene was greatly accelerated (mean latency, 12 wk) with no differences between cohorts lacking one or two *Dmp1* alleles (Fig. 3A). Intriguingly, the latency in the $Dmp1^{+/-}$ or $Dmp1^{-/-}$ strains mimicked that of $Arf^{+/-}$, Eμ-*Myc* transgenic mice (Eischen et al. 1999), consistent with the idea that *Dmp1*

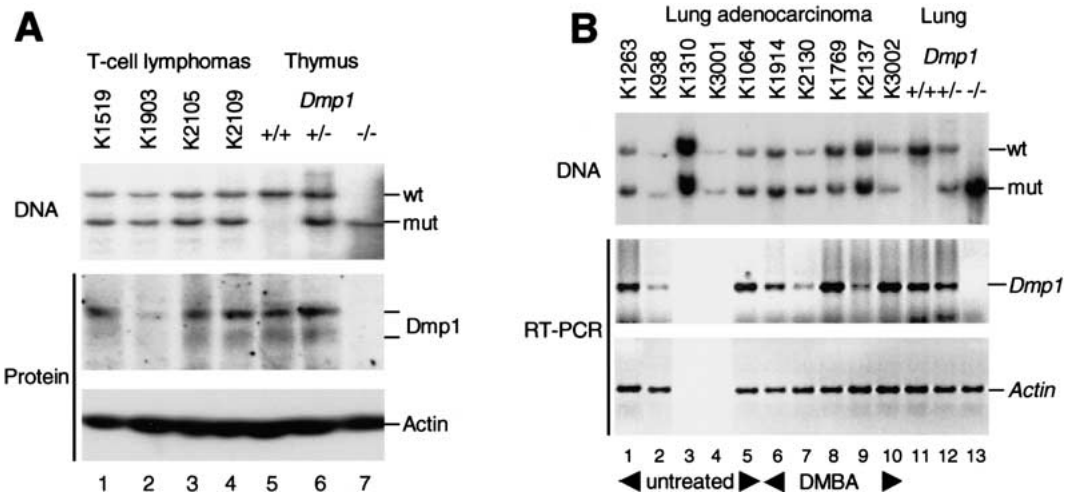

Figure 2. Retention and expression of the wild-type *Dmp1* gene in T-cell lymphomas (*A*) and pulmonary adenocarcinomas (*B*) arising in *Dmp1* heterozygotes. Tumors are designated by K numbers above the lanes. (*B*) (Lanes *1–5*) Results with tumors obtained from untreated animals; (lanes *6–10*) DMBA-treated animals. Protein was detected by direct immunoblotting of tumor cell lysates, and RNA by RT–PCR (not done for tumors K1310 and K3001 in *B*). Actin was used as a control for both procedures to guarantee equal loading. Results with normal tissues of the indicated genotypes appear in the right three lanes of each panel.

loss lowers $p19^{Arf}$ expression (Inoue et al. 2000). Tumors from *Dmp1* heterozygotes retained and expressed the wild-type *Dmp1* allele, and most contained detectable Dmp1 protein (Fig. 3B). Direct nucleotide sequencing of *Dmp1* RT–PCR products from five such tumors identified no mutations in the DNA-binding domain. Gel shift assays confirmed that the residual Dmp1 protein could bind a Dmp1-specific oligonucleotide probe that does not interact with Ets family proteins (Hirai and Sherr 1996) (data not shown). In situ hybridization with an antisense (but not sense) probe confirmed that metastatic lymphomas infiltrating livers of $Dmp1^{+/-}$ mice (Fig. 4C,G) expressed *Dmp1* mRNA, whereas background signal was seen in *Dmp1*-null tumors (Fig. 4D,H). These results provide strong evidence that *Dmp1* is haplo-insufficient for tumor suppression.

We then evaluated the frequency of *p53* mutations and *Arf* loss in Eμ-*Myc* lymphomas. The *p53* gene was retained in all Eμ-*Myc*-induced lymphomas, regardless of their *Dmp1* status (see Supplemental Materials, part D). However, both p53 and $p19^{Arf}$ were grossly overexpressed in 6 of 27 $Dmp1^{+/+}$ tumors (22% incidence) that were suitable for analysis (see Supplemental Materials, part E). These patterns of protein expression are typical of mutant forms of p53, which neither transcriptionally activate *Mdm2* to trigger p53 destruction (Haupt et al. 1997; Kubbutat et al. 1997) nor repress *Arf* transcription (Kamijo et al. 1998; Stott et al. 1998). Seven other $Dmp1^{+/+}$ lymphomas (26%) sustained biallelic *Arf* deletions (see Supplemental Materials, part D). These results closely agree with those of a previous independent study in which *p53* mutations and *Arf* deletions were observed at frequencies of 28% and 24%, respectively, in Eμ-*Myc*-induced lymphomas (Eischen et al. 1999).

In contrast, only 1 of 22 Eμ-*Myc*, $Dmp1^{-/-}$ lymphomas and 3 of 43 Eμ-*Myc*, $Dmp1^{+/-}$ tumors expressed mutant p53 (see Supplemental Materials, part E). The *Arf* gene was retained in all but one of the Eμ-*Myc*, $Dmp1^{-/-}$ tumors and in all but three of the Eμ-*Myc*, $Dmp1^{+/-}$ cases. Therefore, the combined frequencies of *p53* mutation and *Arf* deletion in the $Dmp1^{-/-}$ and $Dmp1^{+/-}$ cohorts were 9% and 14%, respectively, versus 48% in $Dmp1^{+/+}$ littermates. These results provide strong genetic evidence that loss of even a single *Dmp1* allele alleviates the selection for *p53* mutation and *Arf* loss that otherwise occurs during Eμ-*Myc*-induced lymphomagenesis.

Dmp1 loss did not significantly affect the patterns of Mdm2 protein expression in tumors that maintained Arf and p53 function. In those $Dmp1^{+/+}$, $Dmp1^{+/-}$, and $Dmp1^{-/-}$ Eμ-*Myc*-induced lymphomas that lacked evidence of *p53* mutation or *Arf* loss, full-length Mdm2 isoforms were detected in 15%, 14%, and 20% of the respective cases (data not shown), similar to the frequency (16%) observed earlier (Eischen et al. 1999). Therefore, although Mdm2 overexpression may potentially down-

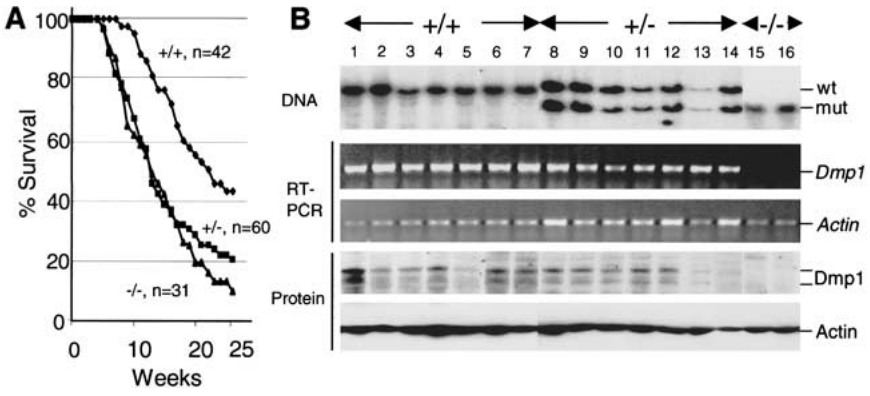

Figure 3. Tumor free survival (*A*) and *Dmp1* status (*B*) of Eμ-*Myc* transgenic animals of the indicated *Dmp1* genotypes. *Dmp1* RNA and protein were detected by RT–PCR and immunoblotting, respectively, using actin as a control for loading.

regulate p53 in a subset of tumors, these effects appear to be independent of *Dmp1* status.

Conclusions and implications

It is now clear that virtually all *Dmp1*-null mice develop cancers of many histological types in their second year of life. Despite the long latency before detection, many such tumors (e.g., pulmonary adenocarcinomas, hepatomas, lymphomas, sarcomas) were highly malignant. *Dmp1* heterozygotes are also highly tumor-prone, whether exposed to carcinogens or irradiated, or left untreated. Notably, in virtually all cases where we could examine relatively pure tumor samples for *Dmp1* DNA, RNA, and protein expression, we failed to obtain evidence that the wild-type *Dmp1* allele was lost, silenced, or mutated. This was particularly evident in Eμ-*Myc* lymphomas arising in $Dmp1^{+/-}$ mice in which the wild-type *Dmp1* allele was retained and shown, by several independent criteria, to be expressed. Although these data do not exclude the possibility that the wild-type *Dmp1* allele can be lost or its expression silenced by other mechanisms, we can fairly conclude that *Dmp1* is haplo-insufficient for tumor suppression. Therefore, in principle, *Dmp1* loss may contribute to certain human monosomy-7 disorders (Bodner et al. 1999).

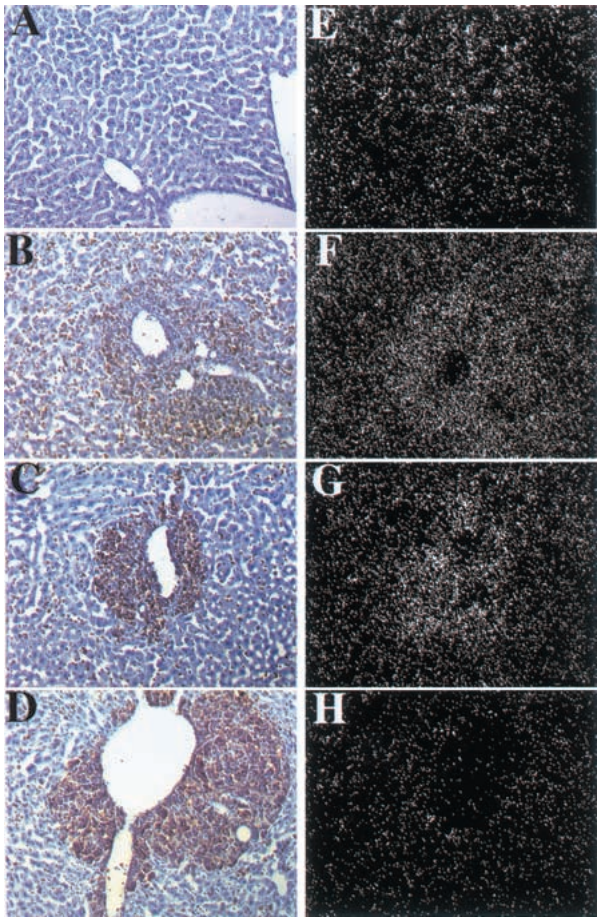

Figure 4. Detection of *Dmp1* RNA by in situ hybridization. Liver cells from $Dmp1^{+/+}$ mice (*A*), stained with hematoxylin (blue) express *Dmp1* RNA (*E*). Malignant lymphoma cells in Eμ-*Myc* transgenic mice, visualized by antibody staining for B220 antigen (brown), surrounded central veins and invaded adjacent liver sinusoids (*B–D*). Regions containing metastatic B-cells in livers from both Eμ-*Myc* $Dmp1^{+/+}$ (*B*) and Eμ-*Myc* $Dmp1^{+/-}$ (*C*) mice revealed increased hybridization signals with a *Dmp1* antisense probe (*F*,*G*), respectively, relative to that in normal liver (*E*), consistent with continued *Dmp1* expression in lymphomas from heterozygous mice. Similar results were obtained in three of three $Dmp1^{+/-}$ animals. Background hybridization with tissues from Eμ-*Myc* $Dmp1^{-/-}$ mice (*H*) was equivalent to that obtained with a control *Dmp1* sense probe (data not shown).

The idea that endogenous Dmp1 can physiologically control *Arf* first emanated from experiments with *Dmp1*-null MEFs, which do not rapidly up-regulate *Arf* upon continuous passage and mimic properties of immortalized *Arf*-null cells (Inoue et al. 2000). A powerful genetic argument that Dmp1 modulates *Arf* gene expression in living animals now stems from studies with Eμ-*Myc*-induced lymphomas. In cultured fibroblasts and in B-lymphocytes in culture and in vivo, Myc acts as a potent *Arf* inducer (Zindy et al. 1998; Eischen et al. 1999; Jacobs et al. 1999a; Schmitt et al. 1999). Selection for *Arf* loss attenuates Myc-induced apoptosis, facilitating immortalization of primary MEFs in culture as well as the appearance of Burkitt-type tumors in Eμ-*Myc* transgenic mice. About 25% of the emerging lymphomas sustain *Arf* deletions, whereas another 25% exhibit *p53* mutations. However, in Eμ-*Myc*-induced $Dmp1^{-/-}$ and $Dmp1^{+/-}$ tumors, the aggregate frequencies of *p53* mutation plus biallelic *Arf* deletion were reduced to 9% and 14%, respectively. Therefore, *Dmp1* loss greatly limits disruption of the latter genes by Myc. Given the susceptibility of $Dmp1^{+/-}$ animals to develop tumors, one prediction is that *Arf* might also be found to be haplo-insufficient for tumor formation under some circumstances. Recent data indicating that $p16^{Ink4a}$ mutation on one chromosome (with *Arf* retention and expression) and monoallelic *Ink4a/Arf* deletion on the other can collaborate in melanomagenesis support this hypothesis (Krimpenfort et al. 2001).

These results raise several other interesting questions. First, the Arf–Mdm2–p53 signaling pathway is disrupted in the vast majority of human cancers, raising the possibility that p53 loss of function, whether partial or complete, is part of the life history of most, if not all, tumor cells. If p53 is indeed dysfunctional at some level in all cancer cells, we might well imagine two broad classes of tumors: (1) those that are disrupted (by mutation, amplification, gene silencing, etc.) in the Arf–Mdm2–p53 axis per se, and (2) those that have sustained mutations in transcription factors (or other modifiers) that modulate the activity of the Arf–Mdm2–p53 pathway. The latter class might include genes such as *Dmp1*, as well as the Arf-negative regulators, *Twist* (Maestro et al. 1999) and *Tbx-2* (Jacobs et al. 2000), that are amplified and overexpressed in particular tumor types. Second, whereas human cancers lacking functional p53 are generally resistant to therapy by ionizing radiation and genotoxic drugs, those that conserve wild-type *ARF*, *Hdm2*, and *p53* genes should retain the ability to respond (Schmitt et al. 1999). This can be tested using the Eμ-*Myc* mouse lymphoma model (Schmitt et al. 2000) and might ultimately be of prognostic significance in human cancers.

Materials and methods

Mice, genotyping, and tumor histology

Cohorts of *Dmp1*-null, heterozygous and wild-type mice (129SvJ × C57BL/6 background) were observed for tumor formation for 24

mo. Genotyping was performed by Southern blotting using *Eco*RV digestion and a 1.1 kb *Eco*47III genomic probe (Inoue et al. 2000). Neonatal treatment of mice with γ-irradiation or DMBA was performed as described (Kamijo et al. 1997). *Dmp1*-null mice were mated with Eμ-*Myc* transgenic mice (C57BL/6 background, kindly provided by Dr. Alan Harris [Walter & Eliza Hall Institute, Melbourne, Australia] and Dr. Charles Sidman [University of Cincinnati, OH]). F_1 *Dmp1*$^{+/-}$, Eμ-*Myc* transgenic mice were crossed with *Dmp1*$^{+/-}$ nontransgenic mice to create *Dmp1*$^{+/+}$, *Dmp1*$^{+/-}$, and *Dmp1*$^{-/-}$ Eμ-*Myc* transgenic animals. All mice were observed daily and sacrificed when moribund. Tumors from euthanized mice were fixed in formalin, and paraffin-embedded tissues were analyzed by light microscopy and immunohistochemistry as described previously (Inoue et al. 2000).

RNA expression

Total RNA, extracted from dissected tissues, was used as a template for cDNA synthesis, followed by PCR amplification and detection of products using murine *Dmp1*-specific primers. The sequence of murine *Dmp1* primers were sense 5′-CTGTAGCTGAAAGAGTGGGTA-3′; antisense 5′-TGTATTATCTTCCAAGCGGGC-3′. Sequences for β-actin primers were sense 5′-GTGGGCCGCCCTAGGCACCAG-3′; antisense 5′-CTCTTTGATGTCACGCACGATTTC-3′. PCR was performed for 30 cycles for *Dmp1* and 21 cycles for β-actin. Under these conditions, both *Dmp1* and β-actin cDNAs were amplified in quantitative ranges.

Immunoblotting

Protein lysates were prepared from freshly isolated T-cell lymphomas, lung adenocarcinomas, or B-cell tumors from Eμ-*Myc* transgenic mice (Kamijo et al. 1997). Briefly, cells were sonicated 2 × 7 sec after addition of ice-cold buffer (50 mM Tris-HCl at pH 8.0, 120 mM NaCl, 0.5% Nonidet P-40, 1 mM EDTA, 1 mM PMSF, 0.4 U/mL aprotinin, 1 mM NaF, and 0.1 mM Na orthovanadate). Undissolved material was sedimented in a microcentrifuge (4°C, 15 min, 14,000 rpm). Proteins (100 μg/lane) were electrophoretically separated on polyacrylamide gels containing sodium dodecyl sulfate (SDS) and transferred onto nitrocellulose (MSI, Westboro, MA). Sites of protein binding were visualized by optimized direct immunoblotting methods (Zindy et al. 1998) with affinity-purified rabbit polyclonal antibodies to the mouse p19^{Arf} (Quelle et al. 1995) and Dmp1 (Hirai and Sherr 1996) carboxyl termini, or with commercial antibodies to p53 (Ab-7, Calbiochem), Mdm2 (C-18, Santa Cruz Biotech), or actin (C-11, Santa Cruz Biotech).

In situ detection of Dmp1 *mRNA in Eμ-*Myc *lymphoma cells metastatic to liver*

Lymphoma-bearing mice were injected intraperitoneally with ketamine and rompun and perfused intracardially with a fixative containing 4% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.6). Isolated livers were transferred into 25% sucrose in 0.1 M sodium phosphate buffer (pH 7.6), at 4°C for an additional 24 h. Serial sections of 12 μm-thickness cut with a cryostat were mounted on Fisher Super-frost-plus slides and stored at –20°C. To create a *Dmp1*-specific probe for in situ hybridization, an *Avr*II–*Bsu*36I fragment (288 bp) that had been deleted from the genome of *Dmp1*-null mice was cloned into the *Avr*II–*Eco*RV site of a pBluescript vector. In situ hybridization was performed as described (Zindy et al. 1997). Malignant B cells infiltrating liver were detected using a rat monoclonal antibody (1:200 dilution, 15 min) to mouse B cell CD45R/B220 (01121D, Pharmingen), biotinylated rabbit antibody (1:200 dilution, 10 min) to rat IgG (BA-4001, Vector Laboratories), and streptavidin congugated to horse radish peroxidase (K1016, DAKO) followed by a 5-min incubation with chromagen substrate (3,3′ diaminobenzidine tetrahydrochloride; K3466, DAKO). Slides were counterstained with hematoxylin, dehydrated, and coverslipped.

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