Mutation and genomic deletion status of *ataxia telangiectasia mutated* (*ATM*) and *p53* confer specific gene expression profiles in mantle cell lymphoma

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Although mantle cell lymphoma (MCL) frequently harbors inactivated *ataxia telangiectasia mutated* (*ATM*) and *p53* alleles, little is known about the molecular phenotypes caused by these genetic changes. We identified point mutations and genomic deletions in these genes in a series of cyclin D1-positive MCL cases and correlated genotype with gene expression profiles and overall survival. Mutated and/or deleted *ATM* and *p53* alleles were found in 56% (40/72) and 26% (21/82) of the cases examined, respectively. Although MCL patients with inactive *p53* alleles showed a significant reduction in median overall survival, aberrant *ATM* status did not predict for survival. Nevertheless, specific gene expression signatures indicative of the mutation and genomic deletion status of each gene were identified that were different from wild-type cases. These signatures were comprised of a select group of genes related to apoptosis, stress responses, and cell cycle regulation that are relevant to *ATM* or *p53* function. Importantly, we found the molecular signatures are different between cases with mutations and deletions, because the latter are characterized by loss of genes colocalized in the same chromosome region of *ATM* or *p53*. This information on molecular phenotypes may provide new areas of investigation for *ATM* function or may be exploited by designing specific therapies for MCL cases with *p53* aberrations.

cancer | cell cycle | genetics | microarray | signature

Mantle cell lymphoma (MCL) is an aggressive tumor that accounts for ≈6% of all non-Hodgkin lymphoma cases in the U.S., with higher rates in North America (1, 2). Although the median survival of MCL patients is only 3 years, some individuals survive >10 years from the time of diagnosis (2, 3). There is considerable interest in defining the molecular basis for this clinical heterogeneity to develop better prognostic markers and more effective therapies.

MCL corresponds to B cells of the mantle zone of the lymphoid follicles that have acquired distinctive alterations in genes related to cell cycle control and apoptosis (4). The hallmark of these genetic alterations is the t(11;14)(q13;q32) translocation that juxtaposes the *IGH* locus near the *CCND1* gene, resulting in the overexpression of cyclin D1 (5). A subset of MCL cases acquire *p53* mutations, and these patients have a significantly shortened median survival relative to cases with wild-type *p53* (6–8). Interestingly, the *ataxia telangiectasia mutated* (*ATM*) gene, whose product regulates some *p53*-dependent apoptosis pathways, is mutated or deleted in 25–40% of MCL cases (reviewed in refs. 9 and 10). Although preliminary studies suggest that *ATM* mutation status does not have a significant impact on patient survival (7, 11), they may have lacked the statistical power to identify more subtle effects on survival, such as the effect of functional subsets of mutations.

We determined the *ATM* and *p53* genotypes in a large cohort of MCL cases with previous gene expression profiles to further elucidate the relationship between molecular phenotypes and clinical outcomes. Whereas *p53* mutation status correlated with overall survival (OS), *ATM* mutation status did not. Nonetheless, there were gene expression signatures indicative of *ATM* and *p53* mutation status. These results provide insights into the altered molecular processes in MCL harboring mutations in these genes and how they relate to clinical outcomes.

Results

***ATM* Aberrations.** Sequence variants were found throughout the coding region of the *ATM* gene (Figs. 2 and 3, which are published as supporting information on the PNAS web site). We placed these variants into three categories: deleterious mutations, unclassified missense changes, or neutral variants (Tables 3–5, which are published as supporting information on the PNAS web site). We define deleterious mutations as sequence changes that produce a truncated ATM protein or alter amino acids critical for *ATM* function. Unclassified missense changes, which are not present in public databases [i.e., database single nucleotide polymorphism (dbSNP)], refer to amino acid substitutions with an unknown effect on *ATM* activity. Neutral variants refer to previously reported sequence variants (i.e., SNPs in dbSNP, or to silent substitutions, regardless of allele frequency in the general population).

Deleterious point mutations in the *ATM* gene were found in 33.3% (24/72) of MCL cases. These included eight nonsense mutations and 13 insertions or deletions. There were six samples with missense mutations in the PI-3 kinase domain as well as six samples with missense changes outside the PI-3 kinase domain that altered conserved amino acids in mouse and/or the African clawed frog. The presence of wild-type alleles, presumably from normal cells, was detected upon sequencing analysis of all cases with deleterious mutations and/or missense variants.

Genomic deletions of the region containing *ATM* locus (11q22.3) were previously found in 30 of 85 (35.3%) MCL cases (4). A detailed mapping analysis of 11q deletions in MCL demonstrated they are typically >10 Mb in length (12). Of the 72 MCL cases analyzed for both *ATM* point mutations (i.e., any sequence change

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Abbreviations: MCL, mantle cell lymphoma; ATM, ataxia telangiectasia mutated; OS, overall survival.

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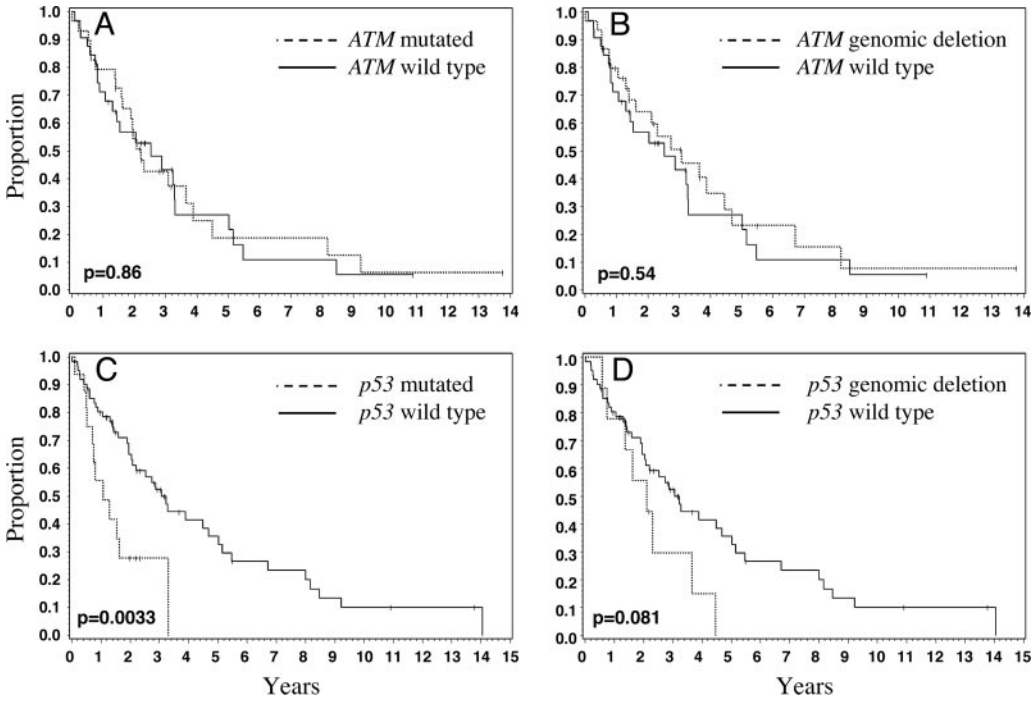

Fig. 1. Overall survival by *ATM* and *p53* mutational status. OS was estimated by the Kaplan–Meier method, and the log-rank test was used to estimate the difference in survival between groups. Here, mutated alleles refer to sequence changes not present in dbSNP, excluding silent changes. Solid black lines indicate cases with wild-type gene of interest, whereas dashed lines indicate cases with aberrations in the gene of interest. The median OS for each genotype is given in parentheses. (*A*) Mutated *ATM* (2.2 years) versus wild-type *ATM* (2.5 years); (*B*) deleted *ATM* (3.1 years) versus wild-type *ATM*; (*C*) mutated *p53* (1.1 years) versus wild-type *p53* (3.1 years); and (*D*) deleted *p53* (2.1 years) versus wild-type *p53*.

not present in dbSNP, excluding silent changes) and genomic deletions, 11 (15.3%) contained only genomic deletions, 16 (20.8%) contained only point mutations, and 13 (18.1%) contained a genomic deletion and a point mutation (see Table 6, which is published as supporting information on the PNAS web site). This last category represents samples that unambiguously have biallelic *ATM* mutations. The weak correlation between the occurrence of deleterious point mutations and deletions in the *ATM* gene (κ statistic = 0.2) indicated the need to use methods for detecting both mutations and deletions to identify all aberrations in the *ATM* gene.

***p53* Aberrations.** A total of 82 cases were screened for hotspot point mutations, polymorphisms, and genomic deletions in the *p53* gene (Tables 7–9, which are published as supporting information on the PNAS web site). We found that 16 of 82 (19.5%) MCL cases contained deleterious *p53* mutations. The mutations included 10 missense mutations, three nonsense mutations, two deletions, and one altered splice site. This is similar to previous reports in MCL, in which missense mutations predominated (6, 8). Genomic deletions of the *p53* locus were previously found in 9.8% (8/82) of MCL cases (4). Here, we find that 6.1% (5/82) had only genomic deletions, 15.9% (13/82) had only point mutations, and 3.7% (3/82) had a genomic deletion and a point mutation. The weak correlation between the occurrence of deleterious point mutations and deletions in the *p53* gene (κ statistic = 0.14) demonstrates the need to use both methods to identify all aberrations in the *p53* gene.

Correlation of *ATM* and *p53* Aberrations. Of the 70 cases analyzed for all aberrations (point mutations and/or genomic deletions) in both genes, 10 (14%) had only *p53* aberrations, 33 (47%) had only *ATM* aberrations, 7 (10%) exhibited aberrations in both genes, and 20 (29%) were wild type for both genes (Table 10, which is published as supporting information on the PNAS web site). *ATM* and *p53* mutations were mutually exclusive in 43 of 50 (86%) cases.

OS. The differences in the median OS of patients with *ATM* point mutations (2.2 years) or patients with *ATM* genomic deletions (3.1 years) from those with only wild-type *ATM* alleles (2.5 years) were not significant ($P = 0.86$ and 0.54, respectively) (Fig. 1 *A* and *B*). The inability of *ATM* mutation status to predict for survival correlates with the mean proliferation signature value (4) of the wild-type versus the *ATM* mutated cases (i.e., 0.02 versus 0.06; see Table 6 and Fig. 4, which is published as supporting information on the PNAS web site). The hypothesis was considered that both alleles of the *ATM* gene may need to be inactivated to affect OS. However, there was no difference in the OS of the 13 cases (3.1 years), where both *ATM* alleles are indisputably inactivated (i.e., via both a point mutation and a genomic deletion) compared with the wild-type group ($P = 0.73$) (data not shown).

In contrast to the results for *ATM* status, there was a significant difference ($P = 0.0033$) in the median OS of patients with *p53* point mutations (1.1 years) compared with those with only wild-type *p53* alleles (3.1 years) (Fig. 1*C*). There was also a trend for worse OS in patients with *p53* genomic deletions (2.1 years) compared with those with only wild-type *p53* alleles (3.1 years; $P = 0.081$) (Fig. 1*D*). The mean proliferation average value (see Table 9 and Fig. 5, which is published as supporting information on the PNAS web site) for tumors with mutant p53 was −0.1 versus 0.3 for the wild-type cases, which places these groups in two different survival quartiles described by Rosenwald *et al.* (4). The median survival for the seven patients with MCL that contained both *ATM* and *p53* aberrations was 1.7 years, which shows no additive effect on survival.

Gene Expression Profiles in Tumors with *ATM* Aberrations. *ATM* was among the 10 genes that were differentially expressed between cases with *ATM* point mutations relative to those containing two wild-type alleles (excluding cases containing genomic deletions) (Table 1 and Fig. 6, which is published as supporting information on the PNAS web site). Thirty-one genes were differentially ex-

Table 1. Gene expression changes in MCL with *ATM* aberrations

Gene symbol	Gene description	Location	Fold change	*P* value
ATM point mutations versus wild-type cases				
EEF1A1	Eukaryotic translation elongation factor 1 α 1	6q14.1	−1.38	1.9×10^{-5}
CYP4B1	Cytochrome P450, subfamily IVB, polypeptide 1	1p34-p12	−1.21	4.1×10^{-4}
C7orf35	DC32 (unknown function)	7q11.23	−1.30	5.4×10^{-4}
MAP3K4	Mitogen-activated protein kinase kinase kinase 4	6q26	−1.24	7.3×10^{-4}
BNIP1	BCL2/adenovirus E1B 19-kDa interacting protein 1	5q33-q34	−1.28	7.7×10^{-4}
ATM	Human ataxia-telangiectasia locus protein	11q22-q23	−1.44	8.7×10^{-4}
CCL20	Chemokine (C-C motif) ligand 20	2q33-q37	−1.52	8.7×10^{-4}
Hs.112482	Similar to glutathione peroxidase	11q14.3	−1.36	8.9×10^{-4}
CDK10	Cyclin-dependent kinase (CDC2-like) 10	16q24	1.27	6.8×10^{-4}
PARVG	Parvin, γ	22q13.2-q13	1.49	7.7×10^{-4}
ATM genomic deletions versus wild-type cases				
*ATM**	Human ataxia-telangiectasia locus protein	11q22-q23	−1.53	1.8×10^{-5}
CCL20	Chemokine (C-C motif) ligand 20	2q33-q37	−1.70†	3.1×10^{-5}
*NPAT**	Nuclear protein, ataxia-telangiectasia locus	11q22-q23	−1.49†	3.6×10^{-5}
RGS16	Regulator of G-protein signaling 16	1q25-q31	−1.53	4.0×10^{-5}
EPHA2	EPH receptor A2	1p36	−1.19	1.5×10^{-4}
EEF1A1	Eukaryotic translation elongation factor 1 alpha 1	6q14.1	−1.33	3.0×10^{-4}
CXCL13	Chemokine (C-X-C motif) ligand 13	4q21	−1.26	4.5×10^{-4}
RPL5	Ribosomal protein L5	1p22.1	−1.35	5.4×10^{-4}
RPS20	Ribosomal protein S20	8q12	−1.25	6.1×10^{-4}
BNIP1	BCL2/adenovirus E1B 19-kDa interacting protein 1	5q33-q34	−1.25	6.7×10^{-4}
XPO1	Exportin 1	2p16	−1.26	6.7×10^{-4}
NOMO2	NODAL modulator 2	16p12.3	−1.22	6.8×10^{-4}
RPL11	Ribosomal protein L11	1p36.1-p35	−1.20	6.9×10^{-4}
PCDH1	Protocadherin 1 (cadherin-like 1)	5q32-q33	−1.58	7.0×10^{-4}
*CASP4**	Caspase 4, apoptosis-related cysteine protease	11q22.2-q22.3	−1.45†	7.1×10^{-4}
CDKN3	Cyclin-dependent kinase inhibitor 3	14q22	−1.27	7.8×10^{-4}
RPS18	Ribosomal protein S18	6p21.3	−1.30†	9.4×10^{-4}
LCK	Lymphocyte-specific protein tyrosine kinase	1p34.3	1.64	2.4×10^{-5}
ZNF207	Zinc finger protein 207	17q11.2	2.20	5.3×10^{-5}
ERCC2	Excision repair, complementation group	19q13.3	1.31	1.8×10^{-4}
ILT7	Leukocyte immunoglobulin-like receptor	19q13.4	1.93	1.9×10^{-4}
ALDH5A1	Aldehyde dehydrogenase 5 family, member A1	6p22.2-p22.3	1.39	2.1×10^{-4}
STAT2	Signal transducer and activator of transcription 2	12q13.3	1.31	2.3×10^{-4}
ITGB7	Integrin, β 7	12q13.13	1.32	3.1×10^{-4}
LILRB1	Leukocyte immunoglobulin-like receptor	19q13.4	1.42	3.3×10^{-4}
LAIR1	Leukocyte-associated Ig-like receptor 1	19q13.4	1.47	3.9×10^{-4}
SFRS7	Splicing factor, arginine/serine-rich 7	2p22.1	1.48	4.1×10^{-4}
ST3GAL1	ST3 β-galactoside α-2,3-sialytransferase 1	8q24.22	1.48	4.3×10^{-4}
CDK10	Cyclin-dependent kinase (CDC2-like) 10	16q24	1.27	7.5×10^{-4}
LBH	Likely ortholog of mouse limb-bud and heart gene	2p23.1	1.37	8.2×10^{-4}
CCM2	Cerebral cavernous malformation 2	7p13	1.42	8.4×10^{-4}

*Located in the 11q22-q23 *ATM* genomic region.
†Average of multiple values. The largest *P* value from random-variance *t* tests for multiple probe sets is provided.

pressed in the tumors with *ATM* genomic deletions relative to those containing wild-type alleles (excludes cases with *ATM* point mutations) (Table 1). They were nonrandomly distributed across the genome, with chromosome 11 showing a significant enrichment (see Fig. 7, which is published as supporting information on the PNAS web site). This was due to three genes (*ATM*, *CASP4*, and *NPAT*) in the 11q22–q23 genomic region that were underexpressed in tumors with *ATM* genomic deletions. Interestingly, *ATM* was the only gene in this region that was differentially expressed in tumors with *ATM* point mutations.

Gene Expression Profiles in Tumors with *p53* Aberrations. Twenty genes were differentially expressed in the tumors containing *p53* point mutations relative to those containing double wild-type alleles (excludes cases with genomic deletions) (Table 2 and Fig. 8, which is published as supporting information on the PNAS web site). Twenty-seven genes were differentially expressed in the tumors with *p53* genomic deletions relative to those containing wild-type alleles (excludes cases with *p53* point mutations) (Table 2). Interestingly, they were nonrandomly distributed across the genome, with chromosome 17 showing a significant enrichment seven genes in the *p53* genomic region (*SPAG7*, *ATP2A3*, *DVL2*, *DPH2L1*, *CENTB1*, *ITGAE*, and *MAP2K4*) (see Fig. 9, which is published as supporting information on the PNAS web site). All seven were underexpressed in tumors with *p53* genomic deletions but not in tumors with *p53* point mutations. However, *p53* was not differentially expressed in cases with either *p53* point mutations or genomic deletions.

Discussion

In agreement with previous studies (7, 9, 11, 13), deleterious or unclassified missense changes in the *ATM* gene were found in 40.3% of MCL cases. Furthermore, we identified deletions in the 11q22-q23 genomic region that covers the *ATM* gene in 24 of 72

Table 2. Gene expression changes in MCL with *p53* aberrations

Gene symbol	Gene description	Location	Fold change	*P* value
p53 point mutations versus wild-type cases				
XPC	Xeroderma pigmentosum, complementation group C	3p25	−1.40*	3.3×10^{-5}
HRB2	HIV-1 rcv-binding protein 2	12q21.1	−1.45	7.3×10^{-5}
PVT1	Pvt1 oncogene homolog, MYC activator (mouse)	8q24	−1.55	3.6×10^{-4}
GBE1	Glucan (1,4-α-), branching enzyme 1	3p12.3	−1.27	5.8×10^{-4}
STK38	Serine/threonine kinase 38	6p21	−1.47	7.6×10^{-4}
GLIPR1	GLI pathogenesis-related 1 (glioma)	12q21.1	−1.41	8.2×10^{-4}
CLK2	CDC-like kinase 2	1q21	−1.24	8.7×10^{-4}
SPAP1	SH2 domain containing phosphatase anchor protein 1	1q21	−1.35	9.1×10^{-4}
PTPN22	Protein tyrosine phosphatase, nonreceptor type 22	1p13.3-p13.1	−1.47	9.5×10^{-4}
MEF2B	MADS box transcription enhancer factor 2	19p12	−1.68	9.9×10^{-4}
BIRC5	Baculoviral IAP repeat-containing 5 (survivin)	17q25	1.68	3.2×10^{-4}
MTHFD2	Methylenetetrahydrofolate dehydrogenase 2	2p13.1	1.39	4.6×10^{-4}
KIT	V-kit Hardy–Zuckerman 4 feline sarcoma oncogene	4q11-q12	1.95	4.6×10^{-4}
CASP7	Caspase 7, apoptosis-related cysteine protease	10q25	1.42	4.7×10^{-4}
CEP2	Centrosomal protein 2	20q11.22-q12	1.44	5.3×10^{-4}
HBZ	Hemoglobin, ζ	16p13.3	1.93	5.7×10^{-4}
PLK1	Polo-like kinase 1 (*Drosophila*)	16p12.1	1.62	6.9×10^{-4}
DAD1	Defender against cell death 1	14q11-q12	1.18	8.2×10^{-4}
JUP	Junction plakoglobin	17q21	1.77	8.7×10^{-4}
ASPM	Asp (abnormal spindle)-like	1q31	1.51	9.8×10^{-4}
p53 genomic deletions versus wild-type cases				
MAP2K4†	Mitogen-activated protein kinase kinase 4	17p11.2	−1.70	2.0×10^{-7}
SPAG7†	Sperm-associated antigen 7	17p13.2	−1.63	1.2×10^{-5}
ATP2A3†	ATPase, Ca++ transporting, ubiquitous	17p13.3	−1.61	1.2×10^{-4}
TAL2	T-cell acute lymphocytic leukemia 2	9q32	−1.47	1.4×10^{-4}
DVL2†	Dishevelled, dsh homolog 2 (*Drosophila*)	17p13.2	−1.60	1.8×10^{-4}
XPC	Xeroderma pigmentosum, complementation group C	3p25	−1.44	1.8×10^{-4}
NFATC2	Nuclear factor of activated T cells	20q13.2–3	−1.41	2.6×10^{-4}
DPH2L1†	Candidate tumor suppressor in ovarian cancer 2	17p13.3	−1.41	3.9×10^{-4}
CENTB1†	Centaurin, β 1	17p13.1	−1.47	5.3×10^{-4}
ITGAE†	Integrin, α E	17p13	−1.39	6.4×10^{-4}
ZFP161	Zinc finger protein 161 homolog (mouse)	18pter-p11.2	−1.38	7.1×10^{-4}
RGS13	Regulator of G protein signaling 13	1q31.2	−2.73	7.7×10^{-4}
TERT	Telomerase reverse transcriptase	5p15.33	−1.39	7.8×10^{-4}
SELL	Selectin L (lymphocyte adhesion molecule 1)	1q23-q25	−2.10	8.3×10^{-4}
POLD1	Polymerase, delta 1, catalytic subunit 125 kDa	19q13.3	1.52	6.5×10^{-5}
CDT1	DNA replication factor	16q24.3	1.62	1.2×10^{-4}
PPP5C	Protein phosphatase 5, catalytic subunit	19q13.3	1.43	1.6×10^{-4}
PLK1	Polo-like kinase 1 (*Drosophila*)	16p12.1	2.00	7.5×10^{-4}
PTP4A3	Protein tyrosine phosphatase type IVA, member 3	8q24.3	1.64	4.0×10^{-4}
BIRC5	Baculoviral IAP repeat-containing 5 (survivin)	17q25	1.90	5.1×10^{-4}
UQCRB	Ubiquinol-cytochrome c reductase-binding protein	8q22	1.39	6.1×10^{-4}
CKS2	CDC28 protein kinase regulatory subunit 2	9q22	1.61*	8.0×10^{-4}
MYC	V-myc myelocytomatosis viral oncogene homolog	8q24.12-.13	2.03	7.4×10^{-4}
PRKAR1B	Protein kinase, cAMP-dependent, reg., type I, β	7pter-p22	1.32	8.3×10^{-4}
HRAS	V-Ha-ras Harvey rat sarcoma viral oncogene	11p15.5	1.37	9.4×10^{-4}
AKAP1	A kinase (PRKA) anchor protein 1	17q21-q23	1.45	9.6×10^{-4}
HN1	Hematological and neurological expressed 1	17q25.1	1.62	9.9×10^{-4}

*Average of multiple values. The largest *P* value from random-variance *t* tests for multiple probe sets is provided.
†Located in the 17p11–17p13 *p53* genomic region.

(33.3%) cases (4). This is somewhat lower than previous studies showing genomic deletion of 11q22-q23 in 50–60% of MCL cases (11, 14–16). This could be due to the sensitivity of our genomic qPCR approach (e.g., given a tumor cell content of 70% in a sample, >30% of the tumor cells in the sample must carry *ATM* genomic deletions in order for the event to be scored).

Because carriers of germ-line *ATM* mutations have not been reported to have a higher incidence of MCL (17), we sought to identify those MCL tumors in which it is certain that both copies of the *ATM* gene were inactivated. Surprisingly, this simultaneous occurrence was lower than expected. Assuming that these *ATM* aberrations are not primarily random events, incurring no selective advantage for cancer cell proliferation and/or survival, there are several possible explanations for the preponderance of single-copy *ATM* mutations. For example, DNA methylation could inactivate expression of the wild-type *ATM* allele, *ATM* may be haploinsufficient, or dominant-negative *ATM* mutations may occur. However, it is unlikely that epigenetic mechanisms silence *ATM* gene expression in all cases, because there are significant differences in *ATM* expression among the wild-type cases and those with *ATM* aberrations. Furthermore, *ATM* promoter hypermethylation was not detected in a study of lymph node biopsies from MCL patients (18).

Last, our assays may have failed to detect sequence changes due to their nature (19) or location (e.g., introns or promoter region) in the *ATM* gene.

In addition, we uncovered point mutations and genomic deletions at the *p53* locus at the expected frequencies. There was a weak correlation between *p53* mutation and genomic deletion status. However, epigenetic phenomenon or false negatives in each analysis may account for a subset of cases in which aberrations affecting both alleles were not found.

Given the interrelationship of *ATM* and *p53* gene function in regulating apoptosis and the cell cycle, we sought to determine whether specific tumors contained at least one inactivated allele (point mutation or genomic deletion) in both genes. In agreement with previous studies, we found no preferential association between *p53* and *ATM* aberrations (7, 11). This indicates there is no strong selective advantage for the acquisition of both *ATM* and *p53* aberrations in MCL.

Consistent with previous reports (7, 11), there was no significant difference in the median OS of patients with tumors having wild-type *ATM* and those with *ATM* point mutations (Fig. 1*A*) or *ATM* genomic deletions (Fig. 1*B*). Interestingly, patients having two mutated *ATM* alleles (one point mutation and one deletion) also showed no significant difference in overall survival relative to patients with only wild-type *ATM* alleles (data not shown). In contrast, patients with aberrant *p53* alleles showed a worse OS relative to wild-type cases (Fig. 1 *C* and *D*), in keeping with our previous observations (6). Furthermore, *p53* mutation status correlates with a higher-proliferation signature average value than wild-type cases, which was determined by expression profiling.

Because *ATM* mutations do not affect the overall survival of MCL patients, it is tempting to question their relevance in MCL development and progression. One could speculate that random point mutations accumulate in the *ATM* gene due to genomic instability. If this were the case, one would predict that missense changes would be randomly scattered throughout the coding region. However, 53.3% of missense changes were located in the last 10% of the *ATM* coding region that contains the highly conserved PI-3 kinase domain. However, there was no difference in OS for this subset of mutations versus mutations located in the rest of the *ATM* sequence (data not shown). This suggests that the point mutations, if somatic in origin, are selected for during lymphomogenesis and are not primarily the consequence of genomic instability in tumors.

Next, to identify the molecular phenotype of *ATM* mutations in MCL, we correlated mutation status with gene expression profiles previously obtained by cDNA microarray analysis (4). Unlike prior analyses, where profiles of mutant *ATM* or *p53* were indistinguishable from profiles of wild-type chronic lymphocytic leukemia (20), we have shown that different profiles are present in mutant cases of MCL. This may be secondary to the larger numbers of cases analyzed and the larger number of genes with usable data in our study. MCL with *ATM* point mutations showed distinct gene expression profiles relative to cases with only wild-type *ATM* alleles. *ATM* was one of 10 genes differentially expressed between these two groups. Several of these genes have functions that are consistent with *ATM* biology. For example, both *CDK10*, a *CDC2*-related protein kinase involved in regulating the G_2/M phase of the cell cycle, and the proapoptotic *BNIP1* transcript are up-regulated in cases with *ATM* point mutations. Consistent with previous hypotheses of *ATM* function (21, 22), *ATM*-deficient MCL cases showed differential expression of genes related to oxygen stress response (e.g., a gene related to glutathione peroxidase, *CYP4B1*, and *MAP3K4*). Finally, the *EEF1A1* gene is down-regulated in *ATM* mutant cases. This is consistent with the repression of a family member (*EEF1D*) in *ATM*-null fibroblasts (23).

Genomic deletions at the *ATM* locus yielded gene expression profiles that could be related to gene dosage. In addition to *ATM*, two other genes in the same 11q22–23 genomic region (*NPAT* and *CASP4*) were down-regulated in these cases. This finding is similar to observations made with U95 Affymetrix arrays in chronic lymphocyte leukemia cases with cytogenetic deletion of 11q22-q23, where seven genes colocalized with *ATM* were down-regulated (24). This confounds our analysis, because it is difficult to determine whether the other genes that are differentially expressed in the cases with *ATM* genomic deletions are due to the loss of *ATM* or its neighbors. Thus, point mutations in and genomic deletions encompassing tumor suppressor genes do not necessarily confer the same molecular phenotypes. Here, only *ATM*, *EEF1A1*, and *CDK10* are differentially expressed in both categories of *ATM* genetic aberrations (point mutations and genomic deletions) relative to cases with only wild-type *ATM* alleles. These form a minimal core group of genes whose expression may be directly related to *ATM* functional integrity.

Next, we determined gene expression profiles related to *p53* mutation status. Interestingly, only a few known *p53* responsive genes (*XPC*, *PLK1*, *BIRC5*, and *GLIPR1*) were differentially expressed in cases with *p53* point mutations relative to cases with two wild-type alleles. *XPC*, involved in nucleotide excision repair, is down-regulated in tumors with *p53* mutations. Thus, this may contribute to the increase in secondary chromosomal abnormalities seen in lymphomas with *p53* mutations. The overexpression of the *PLK1* gene, an important regulator of cell-cycle-related events, is consistent with the decreased OS of *p53*-mutated MCL cases. Elevated *PLK1* expression has been proposed as a prognostic marker and therapeutic target for large numbers of cancers. *BIRC5* (also known as *Survivin*) is overexpressed in *p53*-mutated MCL and strongly associated with the proliferative activity of the tumors and the survival of the MCL patients (25). *GLIPR1*, a tumor suppressor gene that induces apoptosis in human prostate cancer cell lines, was down-regulated in *p53*-mutated cases.

p53 mutated MCL also showed the differential expression of multiple genes relevant to cancer biology. For example, *KIT* and *JUP* are overexpressed in numerous cancers and in *p53*-mutated MCL. *ASPM*, part of a proliferation gene signature related to OS in MCL patients (4), is up-regulated in cases with *p53* point mutations. The induction of the proapoptotic *CASP7* gene and repression of the *DAD1* gene, a negative regulator of apoptosis, could help abrogate the prosurvival effect of *p53* mutations in MCL.

Similar to *ATM*, genomic deletions of the *p53* locus (17p13.1 genomic region) yielded gene expression profiles that could be affected by gene dosage. Strikingly, seven genes in or proximal to 17p11–13 were down-regulated, even though *p53* was not differentially expressed. Similar to *ATM*, the molecular signature of MCL with a deleted *p53* gene differs from that of MCL with *p53* point mutations. Interestingly, expression analyses of *p53*-deleted chronic lymphocyte leukemia (CLL) cases also showed colocalized reductions of expression for 13 genes on the *p53* chromosome segment by using U95 Affymetrix arrays (24). Here, only the *XPC, BIRC5,* and *PLK1* genes are differentially expressed in cases with p53 genomic deletions and/or point mutations relative to those with only wild-type *p53* alleles. These form a minimal core group of genes whose expression may be directly related to *p53* functional integrity in MCL. *XPC*, an important *p53*-regulated gene that is induced by fludaribine therapy of CLL, may be a potential target that could be exploited in MCL (4).

The appearance of distinct clinical phenotypes in MCL cases with *p53* mutations, but not in those with *ATM* mutations, is puzzling, because both genes are important regulators of cell cycle checkpoints and apoptosis. Nevertheless, the gene expression profiles of *ATM*-mutated MCL suggest they have altered biological properties relative to wild-type *ATM* MCL. Indeed, MCL with inactivated *ATM* alleles are associated with increasing numbers of chromosomal imbalances (11). *ATM* aberrations could also play roles in different stages of lymphomogenesis that are not directly related to later clinical manifestations.

Materials and Methods

Patient Materials. Diagnostic biopsies from 92 patients with MCL from the Lymphoma Leukemia Molecular Profiling Project were studied after Institutional Review Board approval (4). Germline status for *ATM* and *p53* is not known for these patients. Cases were chosen that were cyclin D1-positive and where tumor cells comprised the majority (>70%) of cells present on histology review. RNA and DNA were extracted simultaneously from the same piece of tissue. We made use of previously reported gene expression analyses of these tissues by using cDNA microarrays composed of 12,196 clones that interrogate genes that are expressed in lymphoid cells and/or are relevant to cancer or immune function (4).

Statistical Analysis. κ statistics were used to evaluate the agreement between deletion and mutation status in *ATM* and *p53.* A κ statistic >0.75 indicates excellent agreement (26). Survival times were calculated as the time from diagnosis to death or date of last contact. OS distributions were estimated by using the Kaplan–Meier method, and the distributions were compared by using the log-rank test (27, 28).

Differentially expressed genes were identified by using BRB ARRAYTOOLS (15). Random-variance *t* tests were used to find genes that differed significantly between groups (Tables 1 and 2). Genes were considered statistically significant if their *P* value was <0.001. To confirm these results, permutation *P* values for significant genes were computed based on 1,000 random permutations. Only the genes that passed this permutation analysis are listed in Tables 1 and 2 (with all permutation *P* values <0.004 and data not shown). The chromosomal location of the differentially expressed genes was noted, and a χ^2 goodness of fit test was used to test whether the differentially expressed genes were randomly distributed across the chromosomes. Because the absolute values of the fold changes observed in our comparisons typically range from 1.2 to 1.5, caution should be taken when considering the biological significance of a differentially expressed gene.

***ATM* Mutations.** A total of 72 cyclin D1-positive MCL cases were analyzed for mutations on previously described oligonucleotide microarrays (Affymetrix) (7, 29). Six cyclin D1-positive MCL cases previously screened for *ATM* sequence variation by using this platform were included in this study for completeness (7). In principle, the two-color cohybridization experiments and loss of hybridization signal analysis used in this study (see Fig. 2) allowed us to screen for all possible sequence variations in the 62 coding *ATM* exons and their splice junctions (7, 29, 30).

***p53* Mutations.** A total of 82 cyclin D1-positive MCL cases were screened for mutations in *p53* exons 5–8 by denaturing high-performance liquid chromatography using previously described GC clamped primers (6). The optimal assay temperatures were calculated for each exon by using WAVEMAKER software (Transgenomic, Omaha, NE). PCR products with heteroduplex formation were reanalyzed to collect the heteroduplex and homozygous mutant peaks for confirmatory sequencing as described (6).

Genomic Deletions. A total of 85 MCL DNAs were previously analyzed for genomic deletions spanning the *ATM* and *p53* genes using a real-time quantitative PCR technique (4).

Sequencing. Sample exons were amplified, and unincorporated primers and dNTPs were inactivated before sequencing (31) by using the Big Dye Terminator Kit (Applied Biosystems) and halfBD sequencing reagent (Genetix, Boston). Reactions were analyzed on a PRISM 3100 Genetic Analyzer (Applied Biosystems), and SEQUENCHER software (GeneCodes, Ann Arbor, MI) was used to screen for sequence variation. *ATM* amplicons were subcloned and sequenced to identify mutations.

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