Msh6 Protects Mature B Cells from Lymphoma by Preserving Genomic Stability

Jonathan U. Peled,* Rani S. Sellers,† Maria D. Iglesias-Ussel,* Dong-Mi Shin,‡ Cristina Montagna,† Chunfang Zhao,* Ziqiang Li,* Winfried Edelmann,* Herbert C. Morse III,‡ and Matthew D. Scharff*

From the Departments of Cell Biology,* and Pathology,† Albert Einstein College of Medicine, Bronx, New York; and Laboratory of Immunopathology,‡ National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, Maryland

Most human B-cell non-Hodgkin’s lymphomas arise from germinal centers. Within these sites, the mismatch repair factor MSH6 participates in antibody diversification. Reminiscent of the neoplasms arising in patients with Lynch syndrome III, mice deficient in MSH6 die prematurely of lymphoma. In this study, we characterized the B-cell tumors in MSH6-deficient mice and describe their histological, immunohistochemical, and molecular features, which include moderate microsatellite instability. Based on histological markers and gene expression, the tumor cells seem to be at or beyond the germinal center stage. The simultaneous loss of MSH6 and of activation-induced cytidine deaminase did not appreciably affect the survival of these animals, suggesting that these germinal center-like tumors arose by an activation-induced cytidine deaminase-independent pathway. We conclude that MSH6 protects B cells from neoplastic transformation by preserving genomic stability. (Am J Pathol 2010, 177:2597–2608; DOI: 10.2353/ajpath.2010.100234)

DNA mismatch repair (MMR) is a highly conserved process that provides protection from errors made during normal DNA replication and from environmental genotoxic agents.1–4 Heterozygous germline mutations in the human MMR genes MSH2, MSH6, MLH1, or PMS2 lead to hereditary nonpolyposis colorectal cancer, also called Lynch syndrome.5,6 When the normal allele is lost, the resulting neoplastic defect in MMR is often accompanied by deleterious mutations of proto-oncogenes and tumor suppressor genes, as well as instability in microsatellites.

Recent studies have defined an additional familial cancer susceptibility syndrome tentatively named Lynch syndrome III7 that is caused by inheritance of homozygous or compound heterozygous mutations of MMR genes. This syndrome, also referred to as CoLoN (colon tumors or/and leukemia/lymphoma or/and neurofibromatosis),8 childhood cancer syndrome,9 or constitutional MMR-deficiency syndrome,10 is associated with hematomal malignancies in almost half of the known families11 with T-cell lymphomas occurring more commonly than B-cell non-Hodgkin’s lymphomas. Neurological cancers also occur, most often in children but can also appear during the second and third decades of life.

Efforts to understand the normal biology of MMR in mammalian cells, including B lymphocytes in which MMR plays unique roles, have centered on mice with null or...
mutant alleles of MMR genes.\textsuperscript{12,13} MSH2 and MSH6 form a heterodimer that recognizes base-base mismatches arising during DNA replication, as well as mismatches caused by alkylated DNA adducts formed by chemotherapeutic agents or oxidative stress.\textsuperscript{1–4} Msh2\textsuperscript{−/−} mice succumb rapidly to T-cell lymphomas,\textsuperscript{14–16} whereas Msh6-deficient mice die at a somewhat older age, primarily of B-cell lymphomas.\textsuperscript{17} That animals lacking both alleles of an MMR factor develop hematological malignancies is reminiscent of patients with Lynch syndrome III, and these animals provide a potential model system for examining this emerging disease.

B-cell lymphomas in Msh6\textsuperscript{−/−} mice have not yet been characterized in detail, but the observation that MSH6 deficiency is associated primarily with B-cell lymphomas suggests that MSH6 might play a special role in protecting B cells from transformation. This finding is of interest because a high proportion of human B-cell non-Hodgkin’s lymphomas are believed to arise from germinal center (GC) B cells,\textsuperscript{18–20} in which MSH6 directly contributes to the genetic instability required for class switch recombination (CSR) and somatic hypermutation (SHM).\textsuperscript{21–23}

SHM and CSR are mechanisms of antibody diversification that occur in GC B cells.\textsuperscript{13,24} Both are initiated by activation-induced cytidine deaminase (AID), which deaminates cytidines in single-stranded DNA at a very high rate to create uridines that are processed in different ways. When a uridine is converted to a thymidine during replication, this is referred to as a phase 1 mutation. On the other hand, these mutations can be processed by base excision repair or recognized as G:U mismatches and processed by MMR.\textsuperscript{13,34} Paradoxically, the same base excision repair and MMR factors that typically promote genomic stability are recruited to these AID-induced lesions in Ig genes in which they mediate error-prone repair processes that contribute more than half of the mutations that arise in Ig loci. These additional mutations are referred to as phase 2 mutations.\textsuperscript{13} Unlike its heterodimeric partner MSH2, MSH6 physically interacts directly with mismatched bases\textsuperscript{25} and bears within its N-terminal domain a binding motif that probably mediates the interaction of the MMR complex with proliferating cell nuclear antigen (PCNA), which itself recruits error-prone polymerases to Ig variable (V) and switch region sequences.\textsuperscript{26,27} Besides recruiting the subsequent enzymes in MMR, MSH6 can also signal to other cellular programs, such as the apoptosis machinery, although the details of this are not well understood.\textsuperscript{28} These MSH6-specific structural and functional features raise the possibility that it may serve functions that are independent of MSH2 and that are distinct from its paralog MSH3. In MMR, MSH2 can form a heterodimer with either MSH6, which recognizes single-base mismatches, or with MSH3, which recognizes larger mismatches associated with insertions and deletions. Nevertheless, the stable expression of MSH6 requires heterodimerization with MSH2, so most, if not all, of the functions of MSH6 must depend on the presence of MSH2.

Derangements of SHM and CSR are believed to cause many lymphomas. AID- and CSR-mediated mutations in Ig loci and proto-oncogenes can lead to double-stranded DNA breaks and chromosomal rearrangements that deleteriously place certain genes under the control of Ig regulatory sequences.\textsuperscript{29} SHM can be mistargeted to cancer-related genes, such as Bcl6 and Myc, contributing to malignant transformation.\textsuperscript{30} In normal GC B cells, MMR contributes to the error-free repair of AID-induced mutations at many different genomic loci, including some oncogenes, but also carries out error-prone repair of the V and switch regions within the Ig locus as well as to oncogenes such as Bcl6 and a few other genes.\textsuperscript{31} The role of MMR in CSR-mediated pathogenic translocations or aberrant SHM, however, is not known.

Here we describe our characterization of the stage of development from which B-cell lymphomas arise in Msh6\textsuperscript{−/−} mice and our search for the mechanism of neoplastic transformation. We report that the lymphomas arising in Msh6\textsuperscript{−/−} mice have properties of cells at or near the GC stage of B-cell development. In addition, the tumors are quite heterogeneous in their morphology and expression of GC markers. Genomic analysis of expression profiles, karyotype, and copy number alterations are described. We also tested a hypothesis that MSH6 might perform its protective role by repairing AID-induced mutations in oncogenes or tumor suppressors. A genetic cross revealed instead that the events causing malignant transformation of B cells in these mice are not dependent on AID. We conclude that Msh6 protects GC B cells from transformation through its general role in preserving genomic stability rather than through its specific role in contributing to the genomic instability of Ig genes that is associated with SHM and CSR.

Materials and Methods

Mice and Survival Curve

Msh6\textsuperscript{−/−} mice were described previously\textsuperscript{17} and housed in a barrier facility at the Albert Einstein College of Medicine. Aicda\textsuperscript{−/−} mice were the kind gift from Dr. T. Honjo. Because studies in C57BL/6 mice and humans have reported no differences between Aicda\textsuperscript{+/+} and Aicda\textsuperscript{+/−} individuals,\textsuperscript{32–34} Msh6\textsuperscript{−/−}Aicda\textsuperscript{+/−} were included in the cohort of Msh6\textsuperscript{−/−} mice. Although a recent study\textsuperscript{35} reported that loss of a single copy of AID attenuated plasma cell tumors in BALB/c-Bcl-xl transgenic mice, the dosage effects may reflect strain differences.\textsuperscript{36} Survival was analyzed with GraphPad Prism software (GraphPad Software Inc., San Diego, CA). All experiments were approved by the Albert Einstein College of Medicine Institutional Animal Committee.

Histopathology and Immunohistochemistry

Formalin-fixed paraffin-embedded sections were stained with H&E or antibodies as follows. For AID immunohistochemistry (IHC), antigen retrieval was performed in 1
mmol/L EDTA (pH 7.5) in a microwave oven. After standard
locking steps, slides were incubated overnight at
4°C with undiluted supernatant from rat anti-AID hybrida-
oma 328.15 (M. Scharff, manuscript in preparation) and
visualized by standard methods. Antigen retrieval before
B220 (BD Biosciences, San Jose, CA), PNA (Vector Lab-
oratories, Burlingame, CA), CD3 (Dako, Carpintera, CA),
Pax5 (BD Biosciences), MAC387 (Dako), Igκ (Dako), and
IgM (Southern Biotechnology, Birmingham, AL) staining
was performed in a vegetable steamer in Antigen Un-
masking Solution (Vector Laboratories), and Pax5 IHC
was performed using a Mouse-On-Mouse kit (Vector Lab-
oratories). Bcl6 IHC (Santa Cruz Biotechnology, Santa
Cruz, CA) was performed as described.37

Microsatellite Instability, V(D)J Recombination,
and Somatic Hypermutation

Microsatellite instability (MSI) assays were performed
as described in Kuruguchi et al38 and in some cases
were confirmed by direct sequencing. For somatic hy-
permutation (SHM), a 0.5-kb portion of the Ig Jκ2-Jκ4
intron was sequenced as described27 and analyzed
using SHMTool.39

Spectral Karyotype Analysis, Array Comparative
Genomic Hybridization, Expression Profiling

For spectral karyotype analysis, lymphomas were disso-
ciated, filtered, and resuspended in RPMI 1640 com-
pleted with fetal calf serum to 10% and with 4 mmol/L
L-glutamine, 1 mmol/L sodium pyruvate, 100 IU/ml peni-
cillin, 100 μg/ml streptomycin, 0.1 mg/ml gentamicin,
and 55 μmol/L β-mercaptoethanol and supplemented with
100 ng/ml Colcemid (Gibco, Carlsbad, CA), 50 ng/ml
interleukin-4 (R&D Systems, Minneapolis, MN), and 50
μg/ml lipopolysaccharide (Sigma-Aldrich, St. Louis, MO).
After 6 hours of Colcemid treatment, lymphoma cells
were swelled in 75 mmol/L KCl and gradually transferred
to a fixative (3:1 ratio of methanol and glacial acetic acid).
Spectral karyotyping was performed as described40 at
the Genome Imagine Facility of Albert Einstein College of
Medicine. Between five and eight metaphases were an-
alyzed for each tumor, and abnormalities were then de-
fined as described.41

For array comparative genomic hybridization, genomic
DNA from lymphomas and sex-matched C57BL/6J kid-
ney was submitted to Roche NimbleGen (Madison, WI)
for array comparative genomic hybridization on a mouse
whole genome tiling array (385,000 probes). Analysis
was performed as per Roche NimbleGen and with Path-
way Analysis software (Ingenuity Systems, Redwood
City, CA).

For expression profiling, RNA was isolated from frozen
tumor samples as described previously,42 and amplified
RNA from tumors or normal spleen and from reference
RNA (Universal Mouse Reference RNA, Stratagene, La
Jolla, CA) were labeled to Cy3 and Cy5, respectively,
according to the manufacturer’s protocols (Quick-Amp
Labeling Kit, Agilent Technologies, Santa Clara, CA).
Hybridizations were performed onto a customized mouse
Agilent array (Mmeb 4x44K) covering approximately
36,000 genes produced specifically for the National In-
stitute of Allergy and Infectious Diseases Microarray Re-
search Facility. Data from the scanned chips are stored
at the microarray database (mAdb) maintained by the
Center for Information Technology, NIH. Image analysis
and normalization were done with Feature Extraction Soft-
ware (Agilent Technologies). Clustering and principal
component analysis were performed with Genesis43 and
significance analysis of microarrays was applied as
described.44

Results

Msh6-Deficient Mice Develop B-Cell Lymphomas

Although previous studies have reported that Msh6−/−
mice develop B-cell lymphomas, the stage of B-cell dif-
ferentiation reflected by those tumors has not been ex-
amined.17,45,46 We therefore studied new tumors gener-
ated from fully backcrossed Msh6−/− mice. The mice were
regularly monitored and necropsied when mori-
bund. Findings at necropsy included marked spleno-
megaly, frequent enlargement of mesenteric and cervical
lymph nodes, and, less frequently, enlarged mediastinal
nodes. Some mice exhibited hepatic involvement with
multiple small white foci.

By histological criteria,47,48 10 of the 12 cases studied
in detail were diagnosed as B-cell lineage neoplasms
with another two diagnosed as T-cell lymphoblastic lymph-
omas. The B-cell neoplasms included plasmacytomas,
B-cell lymphoblastic lymphomas, immunoblastic lymph-
oma, and follicular lymphoma (Table 1). The degree to
which neoplastic populations ablated normal histological
architecture was variable both between mice and across
different anatomical sites within a given mouse. In some
instances, an entire lymph node was replaced by sheets
of neoplastic cells, whereas in others the tumor cells
comprised a minor population. In addition, some tumors
were associated with large populations of histiocytes.

The most common findings were mixed populations
of neoplastic cells with features of immunoblasts of GC or
early post-GC origin, as well as anaplastic and plasmab-
lastic plasma cells, but only rare mature plasma cells.
Depending on the dominance of a particular cell subset,
these presentations were consistent with diagnoses of
immunoblastic lymphoma and anaplastic and plasmab-
lastic plasmacytoma (APCT)49 (Figure 1, A and E). His-
riotytic infiltrates, including some suggestive of true his-
riotytic sarcoma including multinucleate giant cells
(Figure 1B, arrow) were also common. In cases classified
as lymphoblastic lymphoma (LL), spleen and peripheral
lymph nodes and sometime submandibular and perirenal
lymph nodes were densely populated by lymphoblasts
associated with frequent mitotic figures and a typical
starry sky appearance (Figure 1, C and G) because of the
presence of tingible body macrophages containing
apoptotic bodies (Figure 1G, arrow). The histiocyte-like
neoplastic cells did not stain with the F4/80 antibody (Figure 1F). Finally, one case was diagnosed as follicular B-cell lymphoma with a dense mixture of centrocytes and centroblasts in greatly enlarged splenic follicles (Figure 1, D and H).

Although the B-cell lymphomas identified in the original report of MSH6-deficient mice were from stock on a mixed genetic background,17 a reexamination of these cases identified four APCTs, three LLs, one compound APCT and histiocytic sarcoma, and three diffuse large B-cell lymphomas (DLBCLs) with a variable background of histiocytes (not shown). This finding is consistent with previous reports17,45,46 in which most tumors of Msh6−− mice were histologically of B-cell origin but were frequently associated with large accumulations of normal-appearing or possibly transformed histiocytes.

Table 1. Histological, Immunohistochemical, and Clonal Analysis of Msh6−− Lymphomas

<table>
<thead>
<tr>
<th>Case</th>
<th>Classification*</th>
<th>Clonality†</th>
<th>B220‡</th>
<th>CD3</th>
<th>BCL6</th>
<th>PAX5</th>
<th>PNA</th>
<th>AID</th>
<th>IgM</th>
<th>Igκ</th>
</tr>
</thead>
<tbody>
<tr>
<td>1125</td>
<td>DLBCL</td>
<td>Oligo</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1301</td>
<td>HS + APCT</td>
<td>Mono</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1281</td>
<td>FBL</td>
<td>ND</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>850</td>
<td>LL</td>
<td>Oligo</td>
<td>+</td>
<td>+/-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>480</td>
<td>LS + LL</td>
<td>Mono</td>
<td>+</td>
<td>+/-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>624</td>
<td>HS + PB PCT</td>
<td>Oligo</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
<td>+</td>
</tr>
<tr>
<td>599</td>
<td>LL</td>
<td>Oligo</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>720</td>
<td>LL</td>
<td>Mono</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>981</td>
<td>LL</td>
<td>Oligo</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
<td>+</td>
</tr>
<tr>
<td>164</td>
<td>IB-APCT</td>
<td>Mono</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
<td>+</td>
</tr>
</tbody>
</table>

*Classification based on H&E analysis: DLBCL, diffuse large B cell lymphoma; HS, histiocytic sarcoma; FBL, follicular B cell lymphoma; LL, lymphoblastic lymphoma; PB PCT, plasmablastic plasmacytoma; APCT, anaplastic plasmacytoma; IB-APCT, mixed immunoblastic lymphoma and APCT. Mice 599, 720, 981, and 164 were generated from an Msh6−− by Aicda+/− cross and were heterozygotic at the Aicda locus.

†PCR products from tumors were compared with those from wild-type spleen, in which all possible rearranged alleles were detected. Oligo, oligoclonal; mono, monoclonal; ND, not determined.

‡When both B220 (B cells) and CD3 (T cells) staining was observed, the dominant stain among neoplastic cells was used to assign lineage; two cases scored as T-cell lymphomas are not listed.

Figure 1. Loss of Msh6 leads largely to B-cell lymphomas of diverse morphology. All panels were stained with H&E, except for F, which was stained by F4/80 (brown) and hematoxylin (blue). A: Tumor 1301 is an example of an anaplastic plasmacytoma that effaced the splenic follicular architecture with a uniform population of round cells (E) with ample pale basophilic cytoplasm with round nuclei (plasmacytoid) admixed with histiocytes. B: Tumor 624 is an example of a histiocytic sarcoma in which nuclei are large and oval, and large multinucleated forms are apparent (double arrow). F: The histiocytic-like neoplastic cells do not stain with the F4/80 antibody; a reactive macrophage in the micrograph (arrow) demonstrates effective immunohistochemistry with this antibody. C: Tumor 981 is an example of lymphoblastic lymphoma, with splenic architecture effaced by small dark round cells with uniform round stippled nuclei (G) with one to two generally centrally placed nucleoli and scant cytoplasm (G). Throughout the lesions there were many tingible body macrophages carrying apoptotic bodies (arrow), endowing these tumors with a "starry sky" appearance. D: Tumor 1281 is an example of follicular lymphoma, in which neoplastic B cells have formed nodules composed of generally small cells with round-to-ovoid stippled nuclei, with numerous cells which are histiocytic in appearance (H). Original magnification: ×5 (D), ×10 (A and C), ×10 (B, E–H).
Many Msh6−/− Lymphomas Share Features of GC and Post-GC B Cells

To further investigate the stage of differentiation of MSH6-deficient B-lineage lymphomas, we analyzed the tumors by immunohistochemistry. As suggested by the histological analysis, 10 tumors classified as B-cell lineage lymphomas were confirmed to be of B-cell origin on the basis of B220 staining, and 9 of the 10 were PAX5+ (Table 1). Many cases had prominent populations of CD3+ T cells that were rarely dominant over the B220+ cells and probably represented infiltrating reactive T cells. Variable staining for IgM was observed in four cases and staining for κ light chains was observed in three of the cases diagnosed with populations of plasmablastic and anaplastic plasma cells, as expected from previous studies.40 Three cases (1125, 1281, and 164) were strongly positive for both BCL6 and PAX5, which are highly expressed in normal GC B cells, and they were also positive for expression of two other GC B-cell markers, PNA and AID. One tumor (1301) was positive for BCL6, PAX5, and PNA, and another (981) was strongly positive for BCL6 and weakly positive for PNA and AID. The expression of multiple GC markers suggests that at least six (1125, 1301, 1281, 624, 981, and 164) (Table 1) of the ten tumors were GC-experienced B cells, even though they varied somewhat in their histological appearance.

Microarray expression profiling was performed on three LLs (850, 981, and 480) and one mixed immunoblastic tumor with anaplastic plasmacytoma (IB-APCT) (164). The expression profiles for the LLs were very similar to each other and distinct from those of normal spleens, and the profiles for the LLs differed from that for the IB-APCT by hierarchical clustering (Supplemental Figure S1A, heat map, see http://ajp.amjpathol.org) and principal component analysis (Supplemental Figure S1B, see http://ajp.amjpathol.org) in keeping with their distinct histopathological presentations. To determine whether the pattern of gene expression for the LLs could be associated with a particular normal cell of origin, we compared the pattern for LLs with expression profiles established by microarray analyses of sorted purified reactive T cells. Variable staining for IgM was observed in four cases and staining for κ light chains was observed in three of the cases diagnosed with populations of plasmablastic and anaplastic plasma cells, as expected from previous studies.40 Three cases (1125, 1281, and 164) were strongly positive for both BCL6 and PAX5, which are highly expressed in normal GC B cells, and they were also positive for expression of two other GC B-cell markers, PNA and AID. One tumor (1301) was positive for BCL6, PAX5, and PNA, and another (981) was strongly positive for BCL6 and weakly positive for PNA and AID. The expression of multiple GC markers suggests that at least six (1125, 1301, 1281, 624, 981, and 164) (Table 1) of the ten tumors were GC-experienced B cells, even though they varied somewhat in their histological appearance.

Microarray expression profiling was performed on three LLs (850, 981, and 480) and one mixed immunoblastic tumor with anaplastic plasmacytoma (IB-APCT) (164). The expression profiles for the LLs were very similar to each other and distinct from those of normal spleens, and the profiles for the LLs differed from that for the IB-APCT by hierarchical clustering (Supplemental Figure S1A, heat map, see http://ajp.amjpathol.org) and principal component analysis (Supplemental Figure S1B, see http://ajp.amjpathol.org) in keeping with their distinct histopathological presentations. To determine whether the pattern of gene expression for the LLs could be associated with a particular normal cell of origin, we compared the pattern for LLs with expression profiles established by microarray analyses of sorted purified splenic GC B cells, class-switched memory B cells, and plasma cells.40 As detailed in Supplemental Table 1 (see http://ajp.amjpathol.org), expression of a number of genes was significantly increased or decreased in the LLs. These genes were uniformly over- or underexpressed in all three LLs compared with normal spleen (Supplemental Figure S1, B and C, see http://ajp.amjpathol.org). The results did not reveal a clear relationship of the LLs to a specific normal cell type but did suggest a state of differentiation between GC and plasma cells. This was most easily seen with the “splitting” of canonical GC genes (eg, Aicda increased and Bcl6 decreased) and plasma cell genes (eg, Atf6 increased and Prdm1 decreased).

Msh6−/− Lymphomas Bear Fully Recombined V(D)J Heavy and Light Chain Alleles and Are Monoclonal or Oligoclonal

Although most tumors displayed histological, phenotypic, and molecular features consistent with GC or post-GC B-cell lineage lymphomas, these characteristics are not predictive of clonality. Therefore, we assessed the status of V(D)J recombination of the IgH and κ chain locus by PCR with a panel of degenerate primers that recognize the vast majority of VH and D families and κ light chains.51,52 Fully rearranged alleles were amplified from DNA of all but one (1281) of the lymphomas tested (Table 1). In four tumors (720, 164, 1301, and 480), the presence of a single amplicon was indicative of monoclonality. In the remaining five tumors, several VH-DJκ amplicons or Vκ-DJκ and DJκ amplicons were identified, compatible with the presence of a single clone with two rearranged heavy chain alleles or of a nonproductive DJ and a productive VDJ rearrangement. In some of the tumors that appeared oligoclonal for IgH (850, 624, and 599), a single κ chain rearrangement was detected, effectively ruling out a polyclonal tumor pattern (data not shown). Although we were unable to clearly distinguish monoclonality from oligoclonality in some tumors, polyclonal patterns similar to those seen in wild-type spleens were not observed in any of the cases. Thus, the Msh6−/− B-lineage neoplasms appeared to be monoclonal or oligoclonal.

Microsatellite Instability in Msh6−/− B-Cell Lymphomas

Loss of MSH6 enzymatic activity is associated with MSI in mouse and human tumors.46 To determine whether MSI is also a feature of the tumors studied here, paired DNAs prepared from tumors and tails of individual mice were assayed for MSI using a panel of five mononucleotide and dinucleotide repeats. These markers are among those commonly used to evaluate MSI in mice, and two were selected for their high sensitivity (Table 2).53–56 All tumors showed clear instability for at least one locus; instability was observed in 4 of 12 assays at mononucleotide tracts and in 4 of 7 assays at dinucleotide tracts (Figure 2A and data not shown). These results suggest that MSI could contribute to lymphoma initiation or progression in Msh6−/− mice.

MSI occurs within the coding region of the TGFBR2 gene in human colon cancers57 and in T-cell lymphomas of MSH2-deficient mice.58 Although the (AAAAG)2 sequence in the murine coding region would be expected to provide less opportunity for slippage events than its human counterpart, which is a true mononucleotide repeat, this site has nevertheless been observed to be unstable in murine Msh6−/− lymphomas. Assays of Msh6−/− tumors for MSI in the repeat tracts within the coding sequence of Tgfb2 were negative (Figure 2B), and this result was confirmed by direct sequencing (data not shown).

Chromosomal Changes in Msh6−/− Tumors

Many types of human B-cell non-Hodgkin’s lymphomas exhibit recurrent translocations that place proto-oncogenes under the control of regulatory sequences in the IgH or IgL loci.19 In mouse B-cell lineage neoplasms, T(12;15)
of four tumors, and three were observed in only one tumor. The coordinates in Table 3 are based on the shortest instance of each CNA. The deletion found in tumor 850 that was PAX5/h and PNA/-deficient mice exhibited translocations or microsatellite-unstable tumors (CNA03) on chromosome 11 (Supplemental Figure S2A, see http://ajp.amjpathol.org). No chromosomal translocations were observed. Losses and/or gains of whole chromosomes were observed in most spreads from each tumor and the cell line, but there were no obvious recurrent patterns of gain or loss.

Segmental aneuploidies have been implicated in colon cancers in MMR-deficient mice and microsatellite-unstable human colon cancers.51,52 To test for tumor-specific losses or gains in chromosomal regions beyond the resolution of spectral karyotype, we used array comparative genomic hybridization (Table 3). Excluding physiological V(D)J recombination events within the IgH locus, seven copy number alterations (CNA) involving segmental losses and/or gains in chromosomal regions beyond the resolution of spectral karyotyping were observed. Losses and/or gains of whole chromosomes were observed in most spreads from each tumor and the cell line, but there were no obvious recurrent patterns of gain or loss.

<table>
<thead>
<tr>
<th>Assay</th>
<th>Primer names</th>
<th>Sequence or reference</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microsatellite instability mBat-37 A27</td>
<td>TGFR2-Fwd</td>
<td>5’-GACCTGGAAGCTCTACCTACCCAC-3’</td>
<td>35 cycles of 95°C for 1 minute, 62°C for 30 seconds, 72°C for 30 seconds</td>
</tr>
<tr>
<td>(GT)5 tract of Tgfb2 (AAAG) tract of Tgfb2</td>
<td>TGFR2-Rev2</td>
<td>5’-TACCCCCCTAATGACTGCTTCC-3’</td>
<td>50 cycles of 94°C for 30 seconds, 58°C for 30 seconds, 72°C for 30 seconds</td>
</tr>
<tr>
<td>D7Mit123</td>
<td>UniSTS Accession No. 116680</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D7Mit91</td>
<td>UniSTS Accession No. 131298</td>
<td></td>
<td></td>
</tr>
<tr>
<td>U12225S</td>
<td>A hemi-nested PCR was performed using primers P1 and P256 for 25 rounds of 94°C for 30 seconds, 60°C for 1 minute, and 72°C for 2 minutes, followed by 35 rounds with primers P1 and P3 of 94°C for 30 seconds, 58°C for 30 seconds, and 72°C for 30 seconds</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quantitative RT-PCR Mouse AID</td>
<td>QRT-PCR-mAID.up2</td>
<td>5’-CTGGGAAAAAGCTATGAAA-3’</td>
<td>95°C for 15 minutes followed by 40 cycles of 94°C for 15 seconds, 60°C for 30 seconds, 72°C for 30 seconds</td>
</tr>
<tr>
<td>Mouse GAPDH</td>
<td>QRT-PCR-mAID.down2</td>
<td>5’-GAATAGCTGCTAGGAACTCA-3’</td>
<td></td>
</tr>
<tr>
<td>Somatic hypermutation JH2-JH4 intron</td>
<td>QRT-PCR-mAID.fwd</td>
<td>5’-GACCAATAGCTTCAAGAA-3’</td>
<td>95°C for 5 minutes followed by 35 cycles of 94°C for 30 seconds, 60°C for 1 minute, 72°C for 1 minute</td>
</tr>
<tr>
<td>V(D)J recombinant</td>
<td>Heavy chain locus As per Ehlich et al51</td>
<td></td>
<td>Hot-start PCR</td>
</tr>
<tr>
<td>Light chain V-Jk</td>
<td>Light chain V-Jk As per Yamagami et al52</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Survival of Msh6−/− Mice Is Not Affected by AID Deficiency

Because the highly mutagenic enzyme AID is strongly expressed in normal GC B cells and we observed AID protein in a few of the samples, we asked whether any of the lymphomas exhibited AID-induced SHM. The JH2-JH4 intron of the IgH locus is highly targeted by AID in normal B cells. We amplified and sequenced genomic DNA from this region66 from four tumors. Mutations at frequencies that were higher than the PCR error rate were detected in RWGY hotspot sequences preferentially targeted by AID13 in tumor 624 that had BCL6+ and PAX5+ cells and in tumor 850 that was PAX5+ but BCL6− (Table 1). In contrast, the frequency of mutations was not above the PCR error rate in tumor 1125, which was BCL6+, PAX5+, PNA+, and AID+, nor in tumor 1301, which was BCL6+, PAX5+, and PNA+. The finding of mutations even in
DNA from the same mouse, resolved on sequencing gels, indicate instability in the size of the amplicon between normal tail DNA and tumor translocations in the D7Mit123 in tumors 624 and 981 and of mononucleotide repeat A27 in tumor 599. This instability suggests that AID-induced mutations within the Ig locus of GC B cells, it recruits error-prone repair, leading to the further introduction of phase 2 mutations. Outside of the Ig locus, MMR recruits error-prone repair to only a few genes such as BCL6 but mostly mediates error-free repair of non-Ig genes including oncogenes targeted by AID within GC B cells.\(^{31}\) The mechanisms that regulate this targeting are poorly understood but may involve ubiquitylation of proliferating cell nuclear antigen (PCNA).\(^{26,27}\)

We hypothesized that MSH6 participates in this error-free repair of AID mutations at oncogenes or tumor suppressors and that this is the way in which it might protect B cells from neoplastic transformation. Rather than proceeding to phase 2 mutagenesis, as does in the Ig locus, MSH6 may recruit error-free repair to these AID-induced lesions. If this were true, it would provide an explanation for why B cells are the lineage that becomes malignant in the absence of MSH6, since AID expression is largely limited to B cells. In addition, it might shed light on why Msh6\(^{−/−}\) mice do not develop B-cell lymphomas, because it is a domain within the MSH6 protein that mediates physical recognition and binding of the MSH2/MSH6 complex to G:U mismatches.\(^{25,72}\) Finally, it might also help explain why Msh3\(^{−/−}\) mice do not develop B-cell lymphomas either, because MSH3 does not recognize single base pair mismatches.

To test this hypothesis, we generated mice doubly deficient in MSH6 and AID (Msh6\(^{−/−}\), Aicda\(^{−/−}\)) and compared their survival with that of mice deficient in MSH6 alone for development of disease (Figure 3). If the hypothesis were false, then withdrawal of AID from the Msh6\(^{−/−}\) mice should have no effect on survival. If the hypothesis were true, then removing AID would eliminate the initial hit, the repair function of MMR would be rendered unnecessary, and the mice should exhibit normal survival. Mutant mice of both genotypes began to die at approximately 2 months of age and all were dead at approximately 14 months. Although the median survival for the double knockout mice was 6.0 months versus 8.5 months for Msh6\(^{−/−}\) animals, suggesting a trend toward shorter latency in the absence of AID, the survival curves were not significantly different \((n = 48, \log\text{-}rank \text{ test } P = 0.456).\) The Msh6\(^{−/−}\), Aicda\(^{−/−}\) mice died of lymphomas that histologically and by IHC were largely B-cell LLs

![Figure 2. Msh6\(^{−/−}\) B-cell lymphomas display microsatellite instability. Differences in the size of the amplicon between normal tail DNA and tumor DNA from the same mouse, resolved on sequencing gels, indicate instability not encoded in the germline. A: Examples of instability at dinucleotide repeat D7Mit123 in tumors 624 and 981 and of mononucleotide repeat mBat37 in tumor 624 and of mononucleotide repeat A27 in tumor 599. B: Five Msh6\(^{−/−}\) tumors, as well as a cell line derived from tumor 599, were stable at the (AAAAG)\(_2\) tract within the coding region of Tgfbr2. Stability was also observed at the (GT)\(_3\) repeat tract of the same gene (not shown).](image)

---

Table 3. Copy Number Alterations in Msh6\(^{−/−}\) Lymphomas

<table>
<thead>
<tr>
<th>CNA</th>
<th>Chr</th>
<th>Tumor no.</th>
<th>Band</th>
<th>Start (bp)</th>
<th>Stop (bp)</th>
<th>Length (bp)</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNA01</td>
<td>Chr10</td>
<td>164, 480</td>
<td>qA4</td>
<td>27,519,847</td>
<td>27,577,357</td>
<td>57,510</td>
<td>Loss</td>
</tr>
<tr>
<td>CNA02</td>
<td>Chr7</td>
<td>164, 981</td>
<td>qA3</td>
<td>26,605,452</td>
<td>26,657,288</td>
<td>51,836</td>
<td>Loss</td>
</tr>
<tr>
<td>CNA03</td>
<td>Chr11</td>
<td>164, 480, 981</td>
<td>qA1</td>
<td>3001,791</td>
<td>3099,730</td>
<td>97,939</td>
<td>Loss</td>
</tr>
<tr>
<td>CNA04</td>
<td>Chr14</td>
<td>981</td>
<td>qC2</td>
<td>52,652,778</td>
<td>53,130,162</td>
<td>477,384</td>
<td>Loss</td>
</tr>
<tr>
<td>CNA05*</td>
<td>Chr12</td>
<td>164, 480</td>
<td>qF1, qF2</td>
<td>114,068,720</td>
<td>114,971,457</td>
<td>902,737</td>
<td>Loss</td>
</tr>
<tr>
<td>CNA06</td>
<td>Chr4</td>
<td>480</td>
<td>qC4, qC5</td>
<td>88,688,443</td>
<td>91,879,594</td>
<td>3,191,151</td>
<td>Loss</td>
</tr>
<tr>
<td>CNA07</td>
<td>Chr6</td>
<td>164, 480</td>
<td>qF3</td>
<td>129,938,558</td>
<td>130,203,359</td>
<td>264,801</td>
<td>Loss</td>
</tr>
<tr>
<td>CNA08</td>
<td>Chr11</td>
<td>164</td>
<td>qA1, qA2</td>
<td>11,201,260</td>
<td>16,186,605</td>
<td>4,985,345</td>
<td>Gain</td>
</tr>
</tbody>
</table>

CNA, arbitrary identification assigned to the alterations in this study; Chr, chromosome; Tumor no., the tumors that exhibited the alteration; Start, Stop, and Length coordinates are based on mouse genome assembly February 2006 (mm8); chromosomal bands are from the UCSC Genome Browser.

* This deletion occurred in the IgH locus.
GC B cells. MSH2/MSH3, is important for SHM and CSR in normal lesions in the Ig locus are single-base G:U mismatches but interestingly, loss of MSH3 does not affect lym-
ing cell nuclear antigen (PCNA), and is likely to mediate binding activity, mediates the interaction with proliferat-
tionalredundancy between them.21,79 That AID-generated active types of DNA lesions, there is in fact some func-
tions or insertions are recognized by a heterodimer stretches of mismatches or loops resulting from dele-
MSH2/MSH6 heterodimer, both smaller and larger single mismatched base pairs are recognized by the mediates the interaction with proliferating cell nuclear antigen (PCNA), and is likely to mediate other protein-protein interactions as well.76–78 Whereas single mismatched base pairs are recognized by the MSH2/MSH6 heterodimer, both smaller and larger stretches of mismatches or loops resulting from dele-
ions or insertions are recognized by a heterodimer formed by MSH2 and MSH3. Whereas MSH3 and MSH6 compete for interaction with MSH2 to recruit downstream MMR pathway members to their respective types of DNA lesions, there is in fact some func-
tional redundancy between them.21,79 That AID-generated lesions in the Ig locus are single-base G:U mismatches provides an explanation for why MSH2/MSH6, and not MSH2/MSH3, is important for SHM and CSR in normal GC B cells. Msh3−/− mice are not especially prone to developing neoplasms. Doubly deficient Msh3−/− Msh6−/− mice have shorter survival and increased intest-
tinal tumor development compared with Msh6−/− mice, but interestingly, loss of MSH3 does not affect lym-
phomagenses.80,81 This observation suggests that the role MSH6 plays in protecting B cells from neoplastic transformation or progression is distinct from MSH3 and depends on its ability to recognize single-base mismatches.

In addition to the correction of mispaired bases, MMR is involved in the processing of DNA damage and the subsequent induction of an apoptotic response.28 An inability to mount an apoptotic response to DNA damage is unlikely to be a factor in the genesis of the B-cell lymphomas described here, since mice with a point mut-
ation in MSH6 that markedly interferes with repair func-
tion, but not with apoptotic signaling, also die of B-cell lymphomas with similar latencies.46 Likewise the recently reported ability of MSH2 to suppress Eμ-myelogenous leukemia B-cell lymphomas seems to be unrelated to MMR-depen-
dent apoptosis.82 On the other hand, the unique geno-
tic stress to which GC B cells are subjected18 and the intrinsic MSI exhibited by these cells86 raise the possibil-
ity that MSH6 protects B cells through its traditional en-
zymatic role in mismatch repair.

A protective role for MSH2 and MSH6 in B cells is somewhat paradoxical because the MSH2:MSH6 het-
erodimer contributes ~50% of the AID-dependent muta-
tions in SHM (phase 2) and plays an important role in the recombination events that are involved in CSR,13,24 ac-
tivities that might be expected to contribute to B-cell transformation. Most of the tumors in the Msh6−/− mice were diagnosed histologically as B-cell lineage lympho-
mas belonging to different categories including LLs, DLBLCs with centroblastic or immunoblastic features, PCT with anaplastic, plasmablastic, and plasmacytic fea-
tures, and histiocytic-associated DLBCLs. Histiocyte-rich B-cell lymphomas represents another clinical entity that would benefit from an animal model,84 and it would be interesting in future studies to examine closely the histi-
ocytic component in these tumors. Recent studies sug-
gest that APCT with anaplastic and plasmablastic cytolog-
ies are more closely related to memory B cells than to mature plasma cells or GC B cells, even though they exhibit expression of genes that might traditionally be associated with GC or plasma-cell differentiation pro-
grams (H. Morse, unpublished observations).

In addition, we studied a cell line (designated "599") cloned from one of the oligoclonal B-cell lymphoblastic lymphomas (tumor 599) whose karyotype is listed in Supple-
mmental Figure S2B (see http://ajp.amjpathol.org). We presume it arose from a minor population of the tumor, as its surface molecule phenotype by flow cytometry (B220+, IgM+, and PNA+) was closer to that of normal GC B cells than most of the tumor cells visualized by IHC in the sectional stains of its parent tumor (not shown). This cell line readily formed DLBCL-like tumors in recipient immunosuppressed mice, and AID expression could be induced in culture (not shown). Despite the fact that we were unable to detect ongoing SHM or CSR in this line, these cells may be a potentially useful tool for future studies. Moreover, these observations also suggest that some tumor cells may have expressed GC markers at levels below the sensitivity of IHC or that only minor populations of cells within the tumors have character-
istics of GC cells. This notion is corroborated by the finding that tumor 850 exhibited signature AID-like somatic mutations even though GC markers were not detected by IHC (Table 1 and Supplemental Table 2, see http://ajp.amjpathol.org).

AID-dependent balanced translocations that juxtapose proto-oncogenes to Ig gene regulatory elements are the initiating events in mature B-cell neoplasms of humans and mice as well as mouse plasmacytomas. By spectral karyotype, the B-cell lineage tumors of MSH6-deficient mice did not bear chromosomal translocations, although chromosomal aneuploidies were common. Similar abnormalities have been observed in embryonic fibroblasts from mice deficient in MSH2 and in colon cancers in MMR-deficient mice and humans with microsatellite instability.

Array comparative genomic hybridization, with its greater resolution, did reveal chromosomal regions that were amplified or deleted, and some of these were common to two or three of the four tumors examined (Table 3). Excluding the Igh locus, the seven CNAs identified in this study included the coding regions of 29 genes, of which Ingenuity Pathway Analysis identified 5 (Cdkn2a, Cdkn2b, Kira4, Ikar1, and Grb10) with known roles in cancer. The tumor suppressor genes Cdkn2a (p16INK4a) and Cdkn2b (p15INK4b) are frequently lost in human leukemias and lymphomas, including DLBCLs, and they were deleted in one tumor (480, CNA06) in this study. Ikar1 (Ikaros), a regulator of lymphocyte differentiation that participates in translocations with BCL6 in DLBCLs, was amplified in tumor 164 (CNA08) but its expression was decreased in most of the tumors (Supplemental Table 1, see http://ajp.amjpathol.org). The CNA08 region also includes Grb10, a gene shown to limit in vitro transformation of fibroblasts.

The fifth gene with an annotated cancer function was Kira4 (Ly49D) on chromosome 6 that was deleted in two tumors (164 and 480, CNA07). In vitro studies have suggested that Ly49D inhibits chemotaxis of natural killer cells, a finding of possible relevance to immune surveillance of tumors. The copy number alteration that recurred in three of four tumors (CNA03) in band 11qA1 is in a region syntenic with human chromosome 22q12.2 that includes the human gene neurofibromatosis 2 (NF2). This finding is interesting in light of the clinical overlap between Lynch syndrome III and neurofibromatosis type 1, although it is unclear whether there is a mechanistic link between these two types of neurofibromatosis.

Like the MSI observed in these tumors (Figure 2), the presence of recurrent losses and gains of small chromosomal segments suggests that absence of Msh6 is associated with increased chromosomal instability in B cells. This instability may contribute to or even cause the tumors, as has been reported for MMR-deficient murine colon cancer. However, it is also possible that the CNAs simply accumulated during the course of the neoplastic proliferation. Although MSH6-deficient patients do not exhibit the severe MSI phenotype seen in other MMR-defective hereditary nonpolyposis colorectal cancer, approximately half of MSH6-mutated hereditary nonpolyposis colorectal cancer tumors exhibit low levels of MSI, and a few MSH6-deficient human tumors have been reported to have high levels of MSI. We have previously reported low-to-moderate levels of MSI in murine tumors deficient in MSH6 or carrying inactivating point mutations of MSH6. The partially redundant activities of MSH6 and MSH3 are likely to explain why MSH6-deficient tissues only accumulate low amounts of MSI. These observations are interesting in light of the report that normal GC B cells themselves accumulate instability at microsatellites (see below).

AID is responsible not only for initiating point mutations during SHM as a canonical function but is also involved in noncanonical “off target” SHM activity affecting non-Ig genes that may introduce activating mutations in BCL6, MYC, and other proto-oncogenes. The GC and post-GC stage of differentiation of the tumors from MSH6-deficient mice and the heightened expression of Aicda transcripts seen in the LL prompted us to ask whether AID might contribute to the pathogenesis of these neoplasms, perhaps by introducing phase 1 mutations in oncogenes or tumor suppressors around the genome that are normally repaired in a high-fidelity fashion by MSH6. To address this issue genetically, we generated mice that were both AID-deficient and MSH6-deficient and compared their survival with that of mice deficient for MSH6 alone. The survival of Msh6−/−/Aicda−/− mice was somewhat but not significantly shorter than that of Msh6−/− mice (Figure 3), suggesting that, although AID was not playing a critical role in B-cell neoplasia in Msh6−/− mice, its presence might affect some events late in the transformation process, perhaps even exerting a subtle protective effect. Similar studies of AID in Eμ-myc and MYC transgenic mice also showed no significant differences in survival, although there was not a consensus as to whether tumors in AID-deficient mice were less mature. In Bc6-Myc double transgenic mice, the presence or absence of AID also had no effect on overall mortality, but the tumors of AID-deficient mice were of pre-GC origin compared with the GC origins of the tumors in mice that were AID-sufficient. These findings raise the possibility that in B-cell precursors having already experienced a tumor-initiating event early in development, there may be conditions or factors besides AID that are unique to this lineage and are normally repaired or modulated by MSH6 to protect them from undergoing additional transforming events. Non-neoplastic MMR-deficient GC B cells display high levels of MSI, and GC B cells generate abnormally high numbers of point mutations and double-stranded breaks even in the absence of AID. GC B cells are subjected to genotoxic stress due to their rapid division times and to the suppression of DNA damage-response pathways. In addition, in the absence of AID, GC B cells seem less likely to undergo apoptosis. We speculate that these or other special mechanisms that B cells have evolved have allowed them to tolerate mutagenic and oncogenic events triggered by AID and explain why the AID-sufficient mice survived slightly longer than the doubly deficient mice. Although the molecular nature of these mechanisms is not known, our characterization of Msh6−/− mice supports the notion that B cells are particularly dependent on MMR to main-
tain genomic stability and prevent lymphomagenesis and that this role for MMR is either not dependent on AID or perhaps an alternative scenario in which the absence of MSH6 allows so much instability that the contributions of AID cannot be detected. This study did reveal that AID is not playing a critical role in MSH6-deficient B-cell lymphoma; however, we were unable to clearly explain why loss of MSH6 predisposes to B-cell lymphoma and loss of MSH2 causes T-cell lymphoma. Further examination of the role of MSH6 would provide important insights into the origins of B-cell lymphomas.

Acknowledgments

We authors thank Tasuku Honjo for his generous gift of the Aicda<sup>−/−</sup> mice, Fei Li Kuang and Elena Tosti for experimental assistance and reagents, Lily Chen for assistance with expression profiling, Kan Yang and Steven Brunnert for pathological assistance, John Manis, Elena Avdievich, Sonja Schaetzlein, Radma Mahmoud, and Jacob Glass for technical advice, and Barbara Birshstein and Moshe Sadosky for helpful discussions. Spectral karyotyping was performed in the Genome Imaging Facility of Albert Einstein College of Medicine.

References

70. Gordon MS, Kanegai CM, Doerr JR, Wall R: Somatic hypermutation of the B cell receptor genes B29 (IgH, CD79a) and mb1 (Igk, CD79a). Proc Natl Acad Sci USA 2003, 100:4126–4131
91. Morrione A, Valentinis B, Resnicoff M, Xu S, Baserga R: The role of
90. Edelmann W, Umar A, Risinger JI, Glaub WE, Tindall KR, Barrett JC, Kunkel TA:
89. Elenitoba-Johnson KSJ, Gascoyne RD, Lim MS, Chhanabai M, Jaffe
88. Drexler HG: Review of alterations of the cyclin-dependent kinase
86. Pasqualucci L, Bhagat G, Jankovic M, Compagno M, Smith P,
84. Abramson JS: T-cell/histiocyte-rich B-cell lymphoma: biology, diag-
83. Frey S, Bertocci B, Delbos F, Quint L, Weill J-C, Reynaud C-A:
82. Nepal RM, Tong L, Kolaj B, Edelmann W, Martin A: Msh2-dependent
81. Umar A, Risinger JI, Glaub WE, Tindall KR, Barrett JC, Kunkel TA:
80. Edelmann W, Umar A, Yang K, Heyer J, Kucherlapati R, Lia M,
79. Umar A, Risinger JI, Glaub WE, Tindall KR, Barrett JC, Kunkel TA:
78. Clark AB, Deterding L, Tomer KB, Kunkel TA: Multiple functions for
77. Shell SS, Putnam CD, Kolodner RD: The N terminus of Saccharomy-
76. Shell SS, Putnam CD, Kolodner RD: The N terminus of Saccharomy-
75. Hartley JW, Chattopadhyay SK, Lander MR, Taddesse-Heath, N,
74. Smith DP, Bath ML, Harris AW, Cory S: T-cell lymphomas mask
do faster developing B-lymphoid and myeloid tumors in transgenic
broad haematopoietic expression of MYC. Oncogene 2005,
24:3544–3553
73. Hartley JW, Chattopadhyay SK, Lander MR, Taddesse-Heath, N,
72. Shell SS, Putnam CD, Kolodner RD: The N terminus of Saccharomy-
71. Clark AB, Deterding L, Tomer KB, Kunkel TA: Multiple functions for
70. Edelmann W, Umar A, Yang K, Heyer J, Kucherlapati R, Lia M,
68. de Wind N, Dekker M, Claj N, Jansen L, Klink YV, Radman M,
67. de Wind N, Dekker M, Claj N, Jansen L, Klink YV, Radman M,
66. de Wind N, Dekker M, Claj N, Jansen L, Klink YV, Radman M,
65. de Wind N, Dekker M, Claj N, Jansen L, Klink YV, Radman M,
64. de Wind N, Dekker M, Claj N, Jansen L, Klink YV, Radman M,
63. de Wind N, Dekker M, Claj N, Jansen L, Klink YV, Radman M,
62. de Wind N, Dekker M, Claj N, Jansen L, Klink YV, Radman M,
61. de Wind N, Dekker M, Claj N, Jansen L, Klink YV, Radman M,
60. de Wind N, Dekker M, Claj N, Jansen L, Klink YV, Radman M,
59. de Wind N, Dekker M, Claj N, Jansen L, Klink YV, Radman M,
58. Laguri C, Duband-Goulet I, Friedrich N, Axt M, Belin P, Callebaut I,
57. Laguri C, Duband-Goulet I, Friedrich N, Axt M, Belin P, Callebaut I,
56. Legrand MC, Frey S, Bertocci B: The N-terminal region of Msh6
55. Laguri C, Duband-Goulet I, Friedrich N, Axt M, Belin P, Callebaut I,
54. Laguri C, Duband-Goulet I, Friedrich N, Axt M, Belin P, Callebaut I,
53. Laguri C, Duband-Goulet I, Friedrich N, Axt M, Belin P, Callebaut I,