Mantle cell lymphoma (MCL) is a distinct type of B-cell non-Hodgkin lymphoma that is characterized by a constellation of morphologic, immunophenotypic, and cytogenetic features. It constitutes 3% to 10% of all non-Hodgkin lymphomas in Western countries. Mantle cell lymphoma patients are most often elderly men who present with advanced stage of disease and often have extranodal involvement. Conventional therapy is not effective and the overall prognosis is poor. Mantle cell lymphoma is typically composed of a homogeneous population of small lymphoid cells with scant cytoplasm, clumped chromatin, and irregular nuclear contours. Blastoid and large-cell variants also have been recognized. Typical immunophenotypic features include expression of pan B-cell antigens (CD19, CD20, CD79a), CD5, and CD43, with the lack of CD23 expression. The most characteristic feature of MCL is the t(11;14)(q13;q32). The t(11;14)(q13;q32) juxtaposes the CCND-1 (bcl-1/PRAD1) gene and the immunoglobulin heavy-chain (IgH) gene, resulting in overexpression of cyclin D1. Cyclin D1 interacts with cyclin-dependent kinases (CDKs) to promote cell cycle progression by facilitating phosphorylation and inactivation of retinoblastoma protein (pRB). It is therefore believed that overexpression of cyclin D1 directly contributes to the tumorigenesis of MCL.

Nevertheless, the pathogenesis of MCL is not entirely understood, as previous studies have raised questions regarding the exact pathogenetic role of cyclin D1 in MCL. For example, transfected expression of cyclin D1 in B lymphocytes of transgenic mice does not induce significant alterations in cell cycle progression, nor do these animals have an increased incidence of malignant lymphoma. Also, cyclin D1 protein levels do not correlate with the proliferative activity of MCL cells. More recent data have suggested that defects involving several cell cycle regulatory proteins that control the transition of G1 to S are common in MCL, such as TP53 gene mutations, hypermethylation of the p16INK4a gene, and increased proteasomal degradation of p27Kip1. The pathogenic impact of these defects has not been studied comprehensively.

Pivotal biologic and functional cellular findings have
been discovered by working with human cell lines. The availability of cell lines allows the establishment of experimental models that facilitate understanding of mechanisms, and these lines can be used to test the effects of potential pharmacologic agents. The recent advent of gene transfer techniques, such as the use of adenoviral vectors, has expanded the investigational applications of cell lines. Such techniques have been shown to facilitate evaluation of the functional characteristics of specific genes, their roles in disease, and the possibility of gene therapy. In this regard, the relative lack of understanding of the pathogenesis of MCL can be attributed, at least in part, to the paucity of well-characterized MCL cell lines and the lack of an in vitro study model. Furthermore, the susceptibility of MCL cell lines to transduction by adenovirus vectors is entirely unknown.

With this background, we collected 4 previously developed MCL cell lines and performed extensive characterization, including assessing their susceptibility to transduction by adenovirus vectors. Our goal was to facilitate the establishment of an in vitro model that can be used to study the pathogenesis of MCL.

**METHODS**

**Cell Lines**

The 4 MCL cell lines included in this study are JeKo-1, Mino, SP-53, and Granta 519. JeKo-1 was kindly provided by Tadatsuo Akagi, MD. The cell line Mino was established and characterized in our institution. SP-53 was a generous gift from Masanori Daibata, MD. The cell line Granta 519 was described previously by Jadayel and coworkers and was purchased from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ, Braunschweig, Germany). None of these 4 presumably MCL cell lines have been extensively characterized previously.

With the exception of Granta 519, all cell lines were maintained in RPMI 1640 (Life Technologies, Grand Island, NY) supplemented with 10% heat-inactivated fetal bovine serum (56°C for 30 minutes), penicillin (10000 units/mL; Sigma, St Louis, Mo), streptomycin (10 mg/mL; Sigma), gentamicin (50 μg/mL; Life Technologies), amphotericin B (25 μg/mL; Sigma), and l-glutamine (200mM; 29.2 mg/mL; Life Technologies). For Granta 519, RPMI 1640 was substituted by Dulbecco modified Eagle medium (DMEM). All cell cultures were maintained under an atmosphere of 5% CO₂, 95% oxygen, 5% carbon dioxide, and 98% humidity at 37°C. All cultures were split 3 times weekly and exhibited a doubling time of approximately 24 to 72 hours. Cell counting was performed by Coulter counter, and cell viability was assayed by trypan blue staining.

**Morphologic Features and Immunocytochemistry**

Cells were washed twice in phosphate-buffered saline (PBS) without Ca²⁺ or Mg²⁺. Cytocentrifuge slides were prepared, stained with Wright-Giemsa stain, and examined by light microscopy.

Immunocytochemical staining was performed by a standard technique. Briefly, cytospin preparations were made from each cell line. After air-drying for a minimum of 4 hours and fixation in a mixture of acetone and ethanol (70:30) for 15 minutes, cytospin preparations were rehydrated with PBS, pH 7.6, and incubated with the primary monoclonal antibodies for 1 hour at room temperature. The primary monoclonal antibodies were purchased from commercial sources, and they included cyclin D1 (clone D0-7; 1:50; Dako Corporation, Carpinteria, Calif) and p53 (clone DO-7; 1:50; Dako Corporation, Carpinteria, Calif). Following incubation with the primary monoclonal antibodies, cytospin preparations were treated with an anti-mouse/rabbit biotinylated secondary antibody for 15 minutes. After 3 washes with PBS, avidin conjugated with peroxidase was added for 10 minutes. Diaminobenzidine (25 mg%) in the presence of hydrogen peroxidase was used for the development of a brown color.

**Immunophenotypic Analysis by Flow Cytometry**

Flow cytometric studies were performed for immunophenotypic analysis of the 4 cell lines. The antibodies used in the analysis included anti-CD3, -CD4, -CD5, -CD7, -CD8, -CD10, -CD14, -CD19, -CD20, -CD23, and -CD45; FMC-7; HLA-DR; and κ and λ light chains (Becton Dickinson, San Jose, Calif). A total of 10000 cells from each of the cell lines were analyzed for each marker. The cell lines were assessed using 3-color flow cytometric analysis and a FACScan instrument (Becton Dickinson). Fluorescein isothiocyanate- and phycoerythrin-conjugated IgG1 and IgG2 antibodies were used as negative controls.

**Western Blot Studies**

Western blot analysis was performed using standard techniques. Briefly, the cells were washed in PBS and lysed in a buffer containing 50mM tris-hydrochloride (pH 8.0), 150 mM sodium chloride, 0.1% sodium dodecylsulfate, 1% Nonidet P-40, 1mM phenylmethylsulfonyl fluoride, 5 μg/mL aprotinin, and 5 μg/mL leupeptin. After incubation on ice for 15 minutes, the lysates were subjected to centrifugation at 12000 rpm, and the supernatants were collected. Protein concentration was determined with a protein assay kit (Bio-Rad, Hercules, Calif). Each lane of a 5% to 12% polyacrylamide slab gel received 80 μg of protein. After electrophoresis and transfer to nitrocellulose membrane (Bio-Rad) by electroblotting, blots were probed with specific primary and secondary antibodies and the enhanced chemiluminescence detection system (Amersham, Arlington Heights, III) according to the manufacturer's protocol. The antibodies were used at dilutions of 1:500 to 1:1000. Expression of the following cell cycle- and apoptosis-related proteins were investigated: cyclin D1, cyclin D2, cyclin E, p53, hypophosphorylated form of pRB, pRB, p16(INK4a), p21(WAF1); p27(KIP1); Mcl-1, Bcl-2, Bax, Bcl-x₀, and Bcl-x₁. With the exception of p27(KIP1) (Oncogene Research Products, San Diego, Calif) and Bcl-2 (Dako), all of the antibodies were purchased from Santa Cruz.

**Conventional Cytogenetics and Fluorescence In Situ Hybridization**

Conventional cytogenetic studies were performed using standard methods. Briefly, Colcemid was added to the cells for 1 hour. Thereafter, harvesting was performed according to standard cytogenetic methods. The slides were prepared from the subsequent fixed cell suspension before being trypsinized and stained with Giemsa. Analysis was carried out and karyotypes recorded according to the International Society of Cytogenetics guidelines.

Fluorescence in situ hybridization (FISH) studies were performed using a commercial kit in accordance with the manufacturer's directions (Vysis, Downers Grove, III). Fluorescence in situ hybridization using locus-specific probes for chromosomes 11 and 14 (LSI-IGH for chromosome 14 and LSI-CCND1 for chromosome 11) were applied. Interphases were photographed using a fluorescence microscope (Zeiss, Germany) equipped with the appropriate filter sets.

**Detection of t(11;14)(q13;q32) by Polymerase Chain Reaction**

The t(11;14) junctional sequences were amplified using primers specific for the Bcl-1 locus major translocation cluster region (5'-CCT CTC TCC AAA TTC CTG-3') and the IGH joining region (J₅) (5'-TTT CTC ACC TGA GGA GAC GGT GAC-3'). The expected polymerase chain reaction (PCR) products obtained with Bcl-1/J₅ primers were about 550 to 560 bp. The PCR products were electrophoresed in a 2% agarose gel, transferred to a nylon membrane, and hybridized with a 32P-labeled internal oligonucleotide as described previously.
concentration of approximately 10^4 hours. Additional media was added to the cells to give a final infection (MOI) levels of 10, 50, and 200, and incubated at 37°C for 48 hours after treatment.

The adenoviral transduction efficiency in MCL cell lines was assessed by quantifying the proportion of the cells expressing the green fluorescence protein (Ad-GFP), as previously described.24 Cells were washed with media and concentrated 10-fold, with a final concentration of approximately 10^6 cells/mL. Adenoviral vectors were administered in multiplicity of infection (MOI) levels of 10, 50, and 200, and incubated at 37°C for 4 hours. Additional media was added to the cells to give a final concentration of approximately 10^6 cells/mL. Transduction efficiency was assessed by quantifying the proportion of the cells expressing fluorescence signal, using flow cytometry analysis at 48 hours after treatment.

**RESULTS**

**Clinical Characteristics of the Patients**

The cell line JeKo-1 was developed from the peripheral blood of a 78-year-old woman who presented with MCL in leukemic phase.22 Mino was developed from the peripheral blood of a 78-year-old woman who presented with MCL, an older term for MCL.24 Granta 519 was derived from the peripheral blood of a 58-year-old woman with leukemic MCL, and the neoplastic cells were immortalized with Epstein-Barr virus.25

**Growth Characteristics, Morphology, and Immunocytochemistry**

The characteristics of the 4 MCL cell lines are summarized in Table 1. Figure 1, a, illustrates the cytologic features. JeKo-1 and Mino cells were similar, being large with a moderate amount of deeply basophilic cytoplasm that contained few vacuoles. SP-53 cells were relatively smaller; the cytoplasmic membrane was irregular with occasional projections. Granta 519 cells were large and had a moderate amount of basophilic cytoplasm. In contrast with the other 3 cell lines, Granta 519 showed some cohesiveness. The optimal growth of these cell lines required 10% fetal bovine serum and a minimum of 0.75 to 1 x 10^6 cells/mL.

All cell lines demonstrated positive immunocytochemical staining with cyclin D1 antibodies. Figure 1, b, shows Mino cells. There was notable variation in the intensity of cyclin D1 staining among the individual cells. This phenomenon was also noted in the other 3 cell lines (not shown).

**Evidence of t(11;14)(q13;q32)**

The presence of the t(11;14)(q13;q32) in these cell lines was shown by a variety of techniques. Conventional cytogenetic studies were performed in JeKo-1 and Mino cell lines. Conventional cytogenetics did not demonstrate the classic t(11;14)(q13;q32) in JeKo-1, but rather showed a near-triploid karyotype, as well as the t(10;11;14) abnormality. However, FISH studies confirmed the presence of fusion between the 11q13 and 14q32. Similarly, conventional cytogenetics failed to show the t(11;14)(q13;q32) in Mino. Nevertheless, FISH as well as PCR confirmed the presence of this aberrant fusion gene. The presence of the t(11;14)(q13;q32) in SP-53 and Granta 519 was shown previously.24,25

**Cell Surface Marker Studies by Flow Cytometry**

Table 2 summarizes the expression of cell surface markers in the 4 cell lines, as analyzed by flow cytometry. All 4 MCL cell lines expressed monotypic immunoglobulin

### Table 1. Feature of Mantle Cell Lymphoma Cell Lines*  
<table>
<thead>
<tr>
<th></th>
<th>JeKo-1</th>
<th>Mino</th>
<th>SP-53</th>
<th>Granta 519</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cytology</strong></td>
<td>Single cells</td>
<td>Single cells</td>
<td>Small clumps</td>
<td>Large clumps</td>
</tr>
<tr>
<td><strong>Culture media</strong></td>
<td>RPMI 1640 with 10% FBS</td>
<td>RPMI 1640 with 10% FBS</td>
<td>RPMI 1640 with 10% FBS</td>
<td>DMEM with 10% FBS</td>
</tr>
<tr>
<td><strong>Doubling time, h</strong></td>
<td>24-72</td>
<td>24-72</td>
<td>72</td>
<td>24-72</td>
</tr>
<tr>
<td><strong>S fraction, %</strong></td>
<td>45-50</td>
<td>40-50</td>
<td>35-40</td>
<td>45-50</td>
</tr>
<tr>
<td><strong>Epstein-Barr virus</strong></td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td><strong>Cyclin D1 protein expression</strong></td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td><strong>Evidence of t(11;14)(q13;q32)</strong></td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
</tbody>
</table>
| * FBS indicates fetal bovine serum; DMEM, Dulbecco modified Eagle medium.  
† S fraction as determined by bromodeoxyuridine incorporation detected by flow cytometry (see “Materials and Methods”).
Figure 1. a, Morphologic features of the 4 cell lines. JeKo-1 and Mino cells are large with a high nuclear-cytoplasmic ratio and deeply basophilic cytoplasm. Granta 519 cells are large with moderately abundant, lightly basophilic cytoplasm and show some cohesiveness. SP-53 cells are smaller and show more irregular cytoplasmic membranes with some projections (Wright-Giemsa, original magnification ×1000). b, Immunocytochemical staining of Mino cells with anti-cyclin D1 monoclonal antibodies. There is a significant variation in the intensity of the staining among the individual cells. Similar findings were also noted in the other cell lines (original magnification ×1000).

Table 2. Immunophenotypic Profile of Mantle Cell Lymphoma Cell Lines by Flow Cytometry*

<table>
<thead>
<tr>
<th></th>
<th>JeKo-1</th>
<th>Mino</th>
<th>SP-53</th>
<th>Granta 519</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>CD4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CD5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CD7</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>(P)</td>
</tr>
<tr>
<td>CD8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CD10</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CD14</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CD19</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CD20</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CD23</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CD45</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>FMC-7</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>(P)</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Light chain</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Light chain</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

* P indicates partial expression.

light chain (k in JeKo-1; λ in Mino, SP-53, and Granta 519) and pan B-cell markers, including CD19 and CD20. Coexpression of CD5 was detected in JeKo-1 and Mino, but not in SP-53 and Granta 519. All cell lines expressed FMC-7, although the proportion of positive cells was less in SP-53 and Granta 519. CD23 was absent in JeKo-1 and Mino, but present in SP-53 and Granta 519. Of the T-cell associated markers, CD7 was detected in Mino and a subset of SP-53. Other T-cell–associated markers assessed, including CD3, CD4, and CD8, were negative. CD10 was positive in JeKo-1. The myeloid marker, CD14, was negative in all cell lines.

Expression of G1 Cell Cycle Regulatory Proteins

Table 3 summarizes the expression of various G1 cell cycle regulatory proteins by Western blot analysis, and Figure 2 illustrates some of the results. All 4 cell lines expressed cyclin D1, detected by Western blot. Of the other cyclin D family members, low levels of cyclin D3 were detectable in JeKo-1 and SP-53, whereas cyclin D2 was consistently negative. Cyclin E was highly expressed in all cell lines. Cyclin-dependent kinase inhibitors were differentially expressed among the 4 cell lines: p21WAF1 and p27KIP1 were present in all cell lines, and p16INK4A was detectable only in JeKo-1 and Mino. Retinoblastoma protein was present in all cell lines, and most of the reactivity was found in the slow migrating fraction. A small amount of the hypophosphorylated form of pRB was also present in the slow migrating fraction. p53 protein was present in Mino, SP-53, and Granta 519, with Mino expressing the highest level. JeKo-1 had no detectable p53.

TP53 DNA Sequencing

The TP53 gene was assessed for mutations in each cell line by PCR amplification of exons 5 through 9 and subsequent sequencing of the products. A single mutation in Mino (valine → glycine, codon, exon 5) was detected. No detectable mutations were identified in the other 3 cell lines (Table 3).

Expression of Apoptosis-Related Proteins

Table 4 summarizes the expression of 5 proteins with proapoptotic or antiapoptotic activity by Western blot analysis. The antiapoptotic proteins Bcl-2, Bcl-xL, and Mcl-1 were expressed in all cell lines. The proapoptotic protein Bax was present in all cell lines. Bcl-xS, another proapoptotic protein, was not detected.

Gene Transfer by Adenoviral Vectors

We examined the efficiency of gene transfer by an adenoviral vector carrying the gene encoding for green fluorescence protein (Ad-GFP). As illustrated in Figure 3, Granta 519, SP-53, and JeKo-1 cells showed fluorescence signal at 48 hours after infection with the adenovirus vector. Our results show that 88% of Granta 519 and 82% of SP-53 cells were transduced at an MOI of 50, whereas 75% of JeKo-1 cells were transduced at an MOI of 200. No significant cytotoxicity was detected at an MOI of up to 200 in any of the 3 cell lines susceptible for transduction by the adenovirus. In addition, no significant changes in the cell cycle, as assessed by flow cytometric analysis of 5-bromo-2'-deoxyuridine (BrdU) incorporation, were seen.
Table 3. Expression of the G1 Cell Cycle Regulatory Proteins in Mantle Cell Lymphoma Cell Lines

<table>
<thead>
<tr>
<th>Protein</th>
<th>JeKo-1</th>
<th>Mino</th>
<th>SP-53</th>
<th>Granta 519</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclin D1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cyclin D2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cyclin D3</td>
<td>+ (weak)</td>
<td>-</td>
<td>+ (weak)</td>
<td>-</td>
</tr>
<tr>
<td>Cyclin E</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Retinoblastoma protein</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>c-Myc</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>p16INK4a</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>p21^WAF-1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>p27^kip-1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>p53</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>TP53 sequencing (exons 5–9)</td>
<td>Wild-type</td>
<td>Mutated, exon 5</td>
<td>Wild-type</td>
<td>Wild-type</td>
</tr>
</tbody>
</table>

Figure 2.  
(a) Western blot studies of the different cell cycle regulators and p53. The results are detailed in Table 3. Actin reactivity is equal among the 4 cell lines, denoting similar quantity of protein in each lane.  
(b) The retinoblastoma protein in JeKo-1 was predominantly in hyperphosphorylated/slow-migrating form, as revealed by using antibody reactive with the total retinoblastoma protein (lane A) and antibody specific to the hypo-phosphorylated form (lane B).

Mino cells were not susceptible to transfection by the adenoviral vector.

COMMENT

Mantle cell lymphoma is commonly resistant to conventional therapy and is associated with a relatively poor clinical outcome. This dismal outcome stresses the need to develop novel and effective therapeutic modalities based on a better understanding of the pathogenesis of this disease. Previous studies have documented the existence of defects involving various oncogenes and tumor suppressor genes in MCL, such as cyclin D1, TP53, p16INK4a, and p27^kip-1. Nevertheless, their exact pathogenic roles have not been precisely defined. Research into the pathogenesis of MCL has been hampered by the scarcity of well-characterized MCL cell lines. The lack of an in vitro study model largely limits current studies to the analysis of primary cells from patients, which are often archival. To this end, we collected and comprehensively characterized 4 previously established MCL cell lines.

The identification of these cell lines as MCL was based primarily on the finding of cyclin D1 overexpression and the t(11;14)(q13;q32) in all 4 MCL cell lines. These findings are highly characteristic of MCL. Plasma cell myeloma is the only other B-cell neoplasm that has a relatively high frequency of the t(11;14)(q13;q32) and cyclin D1 overexpression, as it is detected in 15% to 20% of the cases. Overexpression of cyclin D1 at lower levels, not associated with the t(11;14)(q13;q32), has been found in a subset of cases of hairy cell leukemia. The clinical features of the patients from whom these cell lines were established were clearly distinct from those of plasma cell myeloma or hairy cell leukemia.

The immunophenotypic profile of these cell lines is consistent with a mature B-cell immunophenotype. Similar to most MCL cases, 3 of the 4 cell lines expressed λ light chain. The JeKo-1 and Mino cell lines had an immunophenotypic profile similar to that of classic MCL, that is, positive for CD5 and negative for CD23. The immunophenotypic profiles for Granta 519 and SP-53 were unlike that of classic MCL and were negative for CD5 and positive for CD23. These immunophenotypic features do not necessarily imply that these lines are not of MCL origin. Absence of CD5 has been reported previously in MCL, particularly in the more aggressive forms of the disease. CD23 is positive in 5% to 10% of MCL cases. Aberrant
expression of CD7 was seen in SP-53 and Mino cell lines. Although unusual, aberrant expression of T-cell-associated markers has been reported previously in B-cell non-Hodgkin lymphomas, including MCL.\textsuperscript{39} Expression of CD10 in JeKo-1 is also unusual. CD10 is characteristic of follicular lymphoma; however, CD10 in MCL has been previously recognized in a small subset of cases.\textsuperscript{40-42} In addition, cytogenetic and molecular studies performed on JeKo-1 cells did not provide evidence of follicle center cell origin. The discrepancy noted in the immunophenotype between the cell lines and MCL cases could be attributed to the fact that these cell lines might have atypical or distinct biologic features and are perhaps not completely representative of typical cases of MCL.

Our analysis of G1 cell cycle regulatory proteins in these cell lines showed that pRB is predominantly phosphorylated, indicating that cyclin D1 is functional. The expression of pRB has been previously reported in MCL.\textsuperscript{43} Cyclin E is also overexpressed in all cell lines and may also contribute to the inactivation of pRB and its homologs. Cyclin E expression has been shown previously in different types of B-cell non-Hodgkin lymphoma, including MCL.\textsuperscript{44}

Cyclins form complexes with CDKs, which phosphorylate key substrates to initiate DNA synthesis. For example, cyclin D forms a complex with CDK4 and cyclin E interacts with CDK2. One or both of the cyclin D/CDK4 and cyclin E/CDK2 complexes are required for the phosphorylation of pRB and subsequent activation of E2F proteins.

Consistent with a recent report,\textsuperscript{34} we noted that the intensity of cyclin D1 expression varies among individual cells from the same cell line, suggesting that cyclin D1 levels may oscillate during different stages of the cell cycle and/or are posttranscriptionally modulated by some yet-identified mechanisms.

In addition to the overexpression of cyclin D1, recent studies have shown several alterations involving other proteins that participate in regulating progression from the G1 to S phase of the cell cycle in MCL.\textsuperscript{14-16} The present study provides additional evidence for multiple abnormalities in the G1-S regulatory proteins in the 4 cell lines analyzed. Cyclin D3 was weakly expressed in JeKo-1 and SP-53. Cyclin D2 was not detected in all cell lines. In a recent communication, Ott and coworkers\textsuperscript{12} reported that overexpression of cyclin D1 leads to down-regulation of cyclin D3 in MCL. The absence of cyclin D2 and cyclin D3 was previously reported in MCL.\textsuperscript{45,46}

We identified a mutation of the TP53 gene in the Mino cell line. In nonneoplastic cells, p53 induces cell cycle arrest at the G1-S phase. A recent report has shown that p53 plays a critical role in modulating the G2-M transition.\textsuperscript{37} The role of p53 in apoptosis induction is also significant, as it modulates the apoptotic pathways via interaction with Bax, TRAIL, and Fas.\textsuperscript{48} Conceivably, TP53 gene mutations contribute to uncontrolled cellular proliferation and malignant transformation. Nonetheless, p53 expression is limited to only 15% of MCLs, and most of these cases demonstrate blastic morphology and poor progno-
The exact pathogenic role of p53 and its interaction with cyclin D1 and other cell cycle regulatory proteins in MCL require further studies. We also studied 3 CDK inhibitors, p16INK4a, p21Waf1, and p27Kip1. Our results show that p21Waf1 and p27Kip1 are constitutively expressed in all MCL cell lines. p16INK4a is detected only in JeKo-1 and Mino. Previous studies have demonstrated that overexpression or loss of function of these cell cycle regulators plays an essential role in the pathogenesis of MCL. However, the exact function that these regulators fulfill in MCL remains to be further elucidated.

Defects involving the apoptosis pathway appear to play an important role in the pathogenesis of MCL. Hofmann et al recently showed that MCL exhibits alterations in the expression of a number of apoptosis regulatory genes, with an overall pattern favoring promotion of survival via inhibition of apoptosis. It also has been reported that MCL commonly demonstrates high levels of CD40 and low levels of Fas, which may promote cell survival. In our study, we found that the antiapoptotic proteins, Bcl-2, Bcl-xL, and Mcl-1, are constitutively expressed in these cell lines. In contrast, the proapoptotic protein Bax was down-regulated. Bax, another proapoptotic protein, was expressed in all 4 cell lines. A significant role for Bax in the pathogenesis of MCL is doubtful. Recent reports have demonstrated that bax gene mutations are rare in B-cell non-Hodgkin lymphomas, including MCL. However, it is likely that the antiapoptotic signals are more pronounced than the apoptosis-induction pathways in MCL. Identification of the mechanisms that contribute to the relatively high expression of antiapoptotic proteins in MCL cell lines may have clinical relevance, since these mechanisms may serve as potential therapeutic targets. Recently, we identified evidence that STAT3 is constitutively activated in MCL. Since both Bcl-xL and Mcl-1 are targets of STAT3, it is tempting to hypothesize that their expression may be related to the activation of STAT3 in these neoplasms. The utility of these MCL cell lines is further highlighted by the finding that 3 cell lines, JeKo-1, SP-53, and Granta 519, can be readily transduced by adenoviral vectors. Although it has been recognized that hematopoietic cells are generally less susceptible to adenoaviral transduction, several recent reports have demonstrated that adenoaviral transduction of various lymphoma cell lines is achievable. Some of these cell lines are known to possess the receptor for adenoavirus, which may account for their transducibility. Although we did not examine the factors determining the difference in transducibility among the MCL cell lines, it is likely that the presence or absence of these receptors plays an important role. The feasibility of gene transfer in these cell lines could facilitate studies addressing the pathogenic role of various oncogenes and tumor suppressor genes, and their contribution to cell cycle progression, antiapoptotic effects, and resistance to chemotherapeutic agents.

In summary, we have comprehensively characterized 4 MCL cell lines and have summarized their pertinent features. Our results show that these cell lines provide an in vitro model to investigate the molecular defects in MCL. Our observation that 3 of these cell lines are highly transducible by adenovirus vector will open new avenues for exploring the pathogenic role of various oncogenes and tumor suppressor genes, possibly facilitating the development of novel therapeutic regimens in MCL.

References


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