Establishment of the MAVER-1 cell line, a model for leukemic and aggressive mantle cell lymphoma

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Background and Objectives. Mantle cell lymphoma (MCL) cell lines are difficult to generate; only nine lines have been described so far and few of them have been thoroughly characterized.

Design and Methods. We established MAVER-1, a new MCL cell line, obtained from a leukemic MCL harboring both a t(11;14) translocation and a *MYC* rearrangement, and used immunohistochemistry, flow cytometry, molecular biology and cytogenetic techniques in order to characterize the cell line precisely.

Results. By immunohistochemistry and flow cytometry MAVER-1 displayed a classical MCL phenotype (IgM$^+$, λ$^+$, CD5$^+$, CD10$^-$, CD19$^+$, CD20$^+$, CD23$^-$, CD79a$^+$, cyclin D1$^+$) and genetic analysis showed a typical V/D/J rearrangement with naïve mutational status. According to both classic cytogenetic analysis and spectral karyotyping, MAVER-1 harbored the t(11;14) translocation associated with a complex karyotype. Molecular analysis by polymerase chain reactions showed that the t(11;14) breakpoint is within the major translocation cluster. Other important abnormalities of MAVER-1 include *TP53* gene inactivation by a combined mutation of exon 8 and chromosome 17p13 deletion, *ATM* deletion, 8q24 (*MYC*) rearrangement and 8p22 deletion.

Interpretation and Conclusions. The new cell line will be useful for *in vitro* studies regarding MCL pathogenesis and drug sensitivity, as well as a diagnostic control material.

Key words: mantle cell lymphoma, cell line, FISH, cytogenetics, experimental model.

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Mantle cell lymphoma (MCL) is included as a distinct pathologic entity in the REAL and WHO classifications of lymphoid neoplasms.[1] It represents 5 to 10% of all non-Hodgkin's lymphomas, and occurs mainly in middle-aged or elderly adults, with a strong male predominance. MCL is not currently curable by conventional chemotherapy, and the median overall survival at 3.5 years is poor,[2] although more recent treatment modalities may produce a long-term complete remissions.[3] MCL is considered a neoplasm arising from mature, naïve B cells, as indicated by the usual absence of mutations of immunoglobulin genes (IGVH). The typical phenotype of MCL is IgM$^+$, IgD$^{+/-}$, λ>κ, CD5$^+$, CD10$^-$, CD19$^+$, CD20$^+$, CD23$^-$, CD79a$^+$, cyclin D1$^+$. Abnormal cyclin D1 expression is considered the hallmark of MCL, and results from the t(11;14)(q13;q32) translocation that juxtaposes the *CCND1* locus on chromosome 11 to the Igμ enhancer on chromosome 14.[4]

At variance with other lymphoid neoplasms, few cell lines have been established from MCL.[5-14] A recent commentary summarizes the features of eight cell lines presumably derived from MCL from 1986 onwards.[15] Many of these were not fully characterized, not surprisingly considering that the concept of MCL is relatively new. Some cell lines show phenotypes not strictly typical of MCL, such as CD23 expression (Granta-519 and NCEB-1) or lack of CD5 (Granta-519, HBL-2 and SP-49/-50B/-53); others have not been tested for cyclin D1 expression (HBL-2 and SP-49/-50B).

Here we describe the establishment of a novel MCL cell line, called MAVER-1 (for MAntle cell VERona-1). Since the use of a cell line as an experimental model requires its thorough description[16] we have extensively characterized this new cell line by means of flow cytometry, immunohistochemistry, western blotting, polymerase chain reaction (PCR), DNA sequencing, classical cytogenetic analysis, spectral karyotyping (SKY) and fluorescence *in situ* hybridization (FISH).

Design and Methods

Case description

The patient, a 77-year old male, first presented with splenomegaly and lymphocytosis (without superficial lymph node

enlargement) in 1998, underwent bone marrow trephine biopsy at our institution and, after 4 months, splenectomy and lymphadenectomy at another hospital. A peripheral blood sample was sent in January 2003 for characterization by flow-cytometry and western blotting. The patient died a few days later from sepsis.

Establishment of the cell line

Cells were purified from peripheral blood by Ficoll centrifugation (Ficoll-Paque Plus, Amersham, Uppsala, Sweden), washed in phosphate-buffered saline (PBS) and resuspended in RPMI 1640 (Bio-Whittaker, Verviers, Belgium) containing 10% heat-inactivated fetal calf serum (Bio-Whittaker), 2 mM glutamine (Ultraglutamine, Bio-Whittaker) and 50 μg/mL gentamycin. Twenty milliliters of the medium, contained in 75 cm^2 flasks, were incubated at 37°C with 5% CO_2. Cell growth did not require any supplement since cells immediately showed spontaneous proliferative activity. Cells have now been split more than one hundred times and cultured for more than 2 years. In order to determine the growth curve of the cell population cells were seeded at low density (350,000/mL) and counted at 0, 24 , 48 and 72 hours using a cytofluorimeter (FACScan, Becton Dickinson).

Flow cytometry and determination of DNA content

Cells were washed in PBS twice, resuspended at 10^6 cells/mL and incubated with the appropriate antibody (Table 1) for 20 minutes at 4°C, washed in PBS, resuspended and analyzed in a cytofluorimeter (FACScan, Becton Dickinson). For determination of the DNA content, cells were incubated with hypotonic propidium iodide for 30 minutes at room temperature, filtered, and analyzed using appropriate settings.

Immunohistochemistry for tissue sections and cytospin preparations

Immunostaining was performed using the avidin-streptavidin-peroxidase technique (Biogenex, San-Ramon, CA, USA) on both paraffin sections of the primary tumor and ethanol-fixed cytospin preparations of the cell line. The antibody source, dilutions, pretreatment and detection system are summarized in Table 1.

Cytochemistry

Cytochemical stains were performed according to the standard internal procedure manual of the section of Anatomic Pathology of the University of Verona (certified according to the UNI EN ISO 9001:2000 standard – details available from the authors on request).

Western blotting

Protein samples (0.1-0.4 μg/μL) were probed with mouse monoclonal antibodies (Table 1) and horseradish peroxidase-conjugated rabbit anti-mouse secondary antibodies. The blots were developed with the ECL-Plus detection method (Amersham).

Table 1. Antibodies used and their reactivity in MAVER-1.

Method	Antibody	Source	Clone	MAVER-1
FC	CD3/16/56	BD	SK7/B73.1/MY31	Neg
FC	CD4/8	BD	SK3/SK1	Neg
FC	CD5/19	DAKO	DK23/HD37	Pos/Pos
FC	CD7	BD	M-T701	Neg
FC	CD10	PHARMINGEN	HI10a	Neg
FC	CD 14/45	DAKO	TÜK4/T29/33	Neg/Pos
FC	CD19	BD	4G7	Pos
FC	CD20	BD	L27	Pos
FC	CD21	DAKO	1F8	Pos
FC	CD23	BD	EBVCS-5	Neg
FC	CD38	BD	HIT2	Pos
FC	CD79a	SEROTEC	ZL74	Pos
FC	κ	DAKO	Polyclonal	Neg
FC	λ	DAKO	Polyclonal	Pos
FC	IgG	DAKO	Polyclonal	Neg
FC	IgD	DAKO	Polyclonal	Neg
FC	Bcl-2	CALTAG	MHBLC01	Pos
WB	CD3	LABVISION	SP7	Neg
WB	CD5	NOVOCASRA	4C7	Pos
WB	CYC D1	NEOMARKERS	SP4	Pos
WB	P53	DAKO	DO-7	Pos
WB	P21	DAKO	SX118	Neg
WB	BOB-1	SANTACRUZ	C-20	Pos
WB	VIMENTIN	BIOGENEX	V9	Pos
WB	STATHMIN	SIGMA	Polyclonal	Pos
IHC	CD5	NOVOCASTRA	4C7	Neg
IHC	CD20	DAKO	L26	Pos
IHC	CYC D1	NEOMARKERS	SP4	Pos
IHC	P53	DAKO	DO-7	Pos
IHC	P21	DAKO	SX118	Neg
IHC	P16	NEOMARKERS	JC8	Neg
IHC	STATHMIN	SIGMA	Polyclonal	Pos

FC: flow cytometry; WB: western blotting; IHC: immunohistochemistry; Neg: negative; Pos: positive.

DNA and RNA extraction, cDNA synthesis

DNA was obtained from 10^7 cells by standard proteinase K digestion and phenol/chloroform extraction. RNA was extracted using TRIzol (Invitrogen, Paisley, UK), and cDNA was obtained by AMV reverse-transcription (First Strand cDNA synthesis kit, Roche, Mannheim, Germany).

Analysis of VH gene usage

Rearranged *VH* genes were analyzed in both the original peripheral blood sample and the cell line, as described elsewhere.[17] Bands were excised from agarose gels, purified using spin columns (Wizard SV Gel and PCR Cleanup System, Promega, Madison, WI, USA), and cloned into pSTBlue-1 vector (Perfectly Blunt Giga cloning kit, Novagen, Madison, WI, USA). After colony pick-up, bacteria were grown and plasmid DNA was obtained by minipreparations (WizardPlus SV minipreps, Promega), further purified by centrifugal devices (Centricon 100, Millipore, Bedford, MA, USA), and sequenced by a dye terminator reaction (Big Dye v.3.1, Applied Biosystems, Warrington, UK) on a 310 automated sequencer (Applied Biosystems). Data were compared to published germline sequences using IgBLAST (*http://www.ncbi.nlm.nih.gov/igblast/*).

Table 2. Primers used for PCR and sequencing.

Target	*Sequence*	*Use*
T7P	TAATACGACTCACTATAGGGA	Sequencing
U19-MER	GTTTTCCCAGTCACGACGT	Sequencing
P53EX4F	CACCCATCTACAGTCCCCCTT	P53 PCR-SSCP
P53EX4R	GCAACTGACCGTGCAAGTCA	P53 PCR-SSCP
P53EX5F	TCTTCCTACAGTACTCCCCTGCC	P53 PCR-SSCP
P53EX5R	GCTCACCATCGCTATCTGAGC	P53 PCR-SSCP
P53EX6F	GGCCTCTGATTCCTCACTGATT	P53 PCR-SSCP
P53EX6R	AGACCTCAGGCGGCTCATAG	P53 PCR-SSCP
P53EX7F	TGGGCCTGTGTTATCTCCTAGG	P53 PCR-SSCP
P53EX7R	CAAGTGGCTCCTGACCTGGA	P53 PCR-SSCP
P53EX8F	TGGTAATCTACTGGGACGGAACA	P53 PCR-SSCP
P53EX8R	TCCTGCTTGCTTACCTCGCT	P53 PCR-SSCP
P53EX9F	GCAGTTATGCCTCAGATTCACTTTT	P53 PCR-SSCP
P53EX9R	TCCCAAGACTTAGTACCTGAAGGGT	P53 PCR-SSCP
JH	ACCTGAGGAGACGGTGACC	t(11;14) PCR
MCL-2	TTCAGGCCTTGATAGCTCGT	t(11;14) PCR
MCL-4	AATAAGGCTGCTGTACACATCG	t(11;14) PCR
VH1	CTCAGTGAAGGTYTCCTGCAAGGC	VH PCR
VH2	GTCCTGCGCTGGTGAAASCCACAC	VH PCR
VH3	GGGTCCCTGAGACTCTCCTGTGCAG	VH PCR
VH4	CCTGTCCCTCACCTGCRCTGTC	VH PCR
VH5	AAAAAGCCCGGGGAGTCTCTGARGA	VH PCR
VH6	CTGTGCCATCTCCGGGGACAGTG	VH PCR
3'JH 1,2,4,5	ACCTGAGGAGACGGTGACCAGGGT	VH PCR
3' JH3	TACCTGAAGAGACGGTGACCATTGT	VH PCR
3' JH6	ACCTGAGGAGACGGTGACCGTGGT	VH PCR
5'JH1,3,4,5	GGTGACCAGGGTBCCYGGCC	VH PCR
5'JH2	AGTGACCAGGGTGCCACGGCC	VH PCR
5'JH6	GGTGACCGTGGTCCCTTGCC	VH PCR

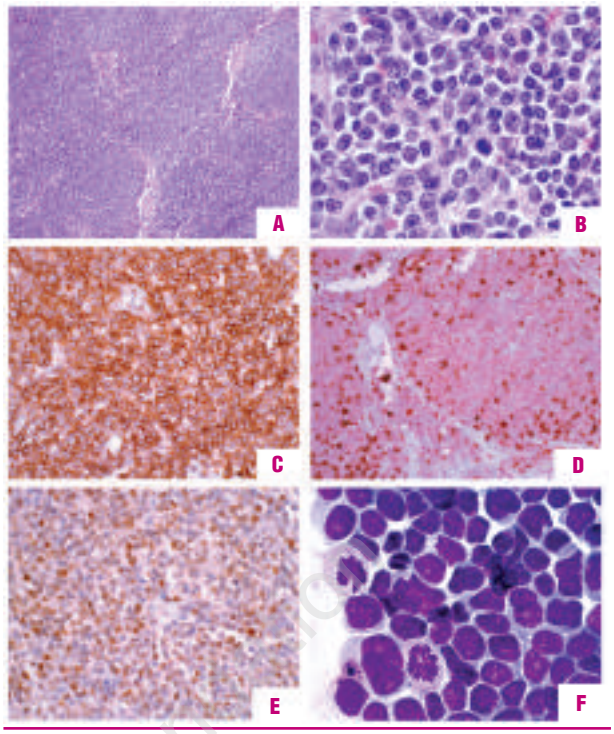

Figure 1. Morphology and immunohistochemistry of the original lymphoma sample and the MAVER-1 cell line. Low power (A) and high power (B) view of the lymph node sample show effacement of the normal architecture due to a nodular and confluent lymphoid infiltrate composed of a spectrum of small, medium-sized and large cells, which are CD20 positive (C), CD5 positive (D) and cyclin D1 positive (E). The cell morphology of MAVER-1 resembles that of the original lymphoma, with a spectrum of cells showing round-ovoid or angulated nuclei (May-Grünwald-Giemsa stain)(F).

Detection of t(11;14) by PCR

Two different primer sets were used (Table 2); one previously described[18] and one newly created on the basis of the published sequence (GenBank accession number X74150). The new primer set was found to be more specific and efficient than those previously described and was therefore used for PCR cloning. Cycling conditions were 94°C for 4'; 94°C for 30", 60.4 to 56°C touch-down (0.4°C decrements at each cycle) for 15", 65°C for 40", repeated 11 times; 94°C for 30", 56°C for 15", 65°C for 40", repeated 26 times; 65°C for 5'. The reaction mix was composed of 1x HotMaster Buffer (Eppendorf AG, Hamburg, Germany), 0.2 μM of each dNTP, 0.2 μM of each primer, 1U HotMaster Taq polymerase (Eppendorf) and 50 ng genomic DNA. PCR products were run on 3% agarose gels containing 0.5 μg/mL ethidium bromide and visualized by UV trans-illumination.

Cloning of the t(11;14) breakpoint

PCR products were purified by spin columns (Wizard SV Gel and PCR Cleanup System, Promega), cloned and sequenced using the same procedures as those used for *VH* gene analysis. The sequence was further analyzed by DNA analysis software (DNASIS Max, Hitachi Software, Hamburg, Germany).

Mutational analysis of TP53

Exons 4 to 9 were screened for mutations by PCR-single strand conformational polymorphism (SSCP) analysis. Fifty nanograms of genomic DNA were amplified with exon-specific primers (Table 2). Amplified products were screened using a thermally-controlled electrophoresis apparatus (GenePhor, Amersham, Uppsala, Sweden) and dried-precast acrylamide gels (GeneGel SSCP Starter Kit, Amersham) as recommended by the manufacturers. Regions corresponding to the shifted bands were sequenced using upstream and downstream primers (Table 2).

Epstein-Barr virus status evaluation

Epstein-Barr virus encoded RNA (EBER) hybridization and LMP-1 immunohistochemical staining were performed on cytoinclusions. EBER *in situ* hybridization was repeated twice, after 6 months and after 2 years of continuous culture. Briefly, cells were spun, washed with PBS, and finally resuspended in 2% low melting point agarose at 38°C, poured into disposable plastic moulds and cooled on ice. Solid agarose blocks were put inside a histological cassette, fixed in 4% buffered formalin for 2h at room temperature and processed routinely as a tissue block. Sections 4μm thick were cut from the block and processed using the EBER PNA probe (DakoCytomation, Glostrup, Denmark) and PNA ISH Detection Kit (DakoCytomation). These experiments were repeated twice after 6 months and 2 years of continuous culture.

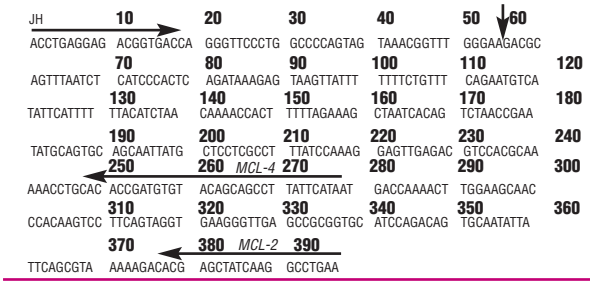

Figure 2. Breakpoint sequence of the t(11;14) translocation. Horizontal arrows: position of the primers. Vertical arrow: putative breakpoint.

Classical cytogenetic analysis

Cell cultures were pulsed with colchicine (10 μg/mL) for 30 minutes before harvesting. For metaphase preparation the cells were treated with a hypotonic solution of 0.075M KCl and fixed in a 3:1 solution of methanol-glacial acetic acid after several washing steps. Cells in suspension were dropped onto ethanol-cleaned glass slides and were allowed to mature for 7-12 days at room temperature. Metaphase chromosomes were stained using a modified trypsin-Giemsa technique. Metaphases were analyzed and evaluated according to the International System for Human Cytogenetic Nomenclature.[19] A chromosomal alteration was regarded as clonal if either two or more cells/metaphases harbored the same structural aberration or chromosomal gains, or if the same chromosome loss was found in at least three different cells/metaphases. Images were captured with a ZEISS Axioskop 2 microscope (ZEISS, Jena, Germany) and evaluated using the IKAROS imaging system (MetaSystems, Altussheim, Germany).

Two-color FISH analysis

FISH was performed using a panel of different locus-specific probes representing the 15 most common genetic alterations in MCL as defined by classical cytogenetic and comparative genomic hybridization analyses of MCL (Table 3). Dual-color FISH with directly labeled probes was performed using commercially available probes, except for alterations affecting *ATM* for which we applied a biotin-dUTP or digoxigenin-dUTP-labeled P1-derived artificial chromosome (PAC) probe specific for *ATM* (PAC ATM-2, LLNLP704O01298Q19),[20] and chromosome bands 1p22, 6q27, 7p15, 8p22, 12q12/13, and 15q21 for which yeast artificial chromosome probes specific for these loci were used, as described elsewhere[21,22] (Table 3). Signals were visualized using a ZEISS Axioskop 2 fluorescence microscope (ZEISS) and illustrations were made using the ISIS imaging system (MetaSystems). At least 100 to 200 intact interphase nuclei per case were evaluated and an aberrant clone was defined according to the cut-off level for each probe. This cut-off was defined using a reference composed of at least

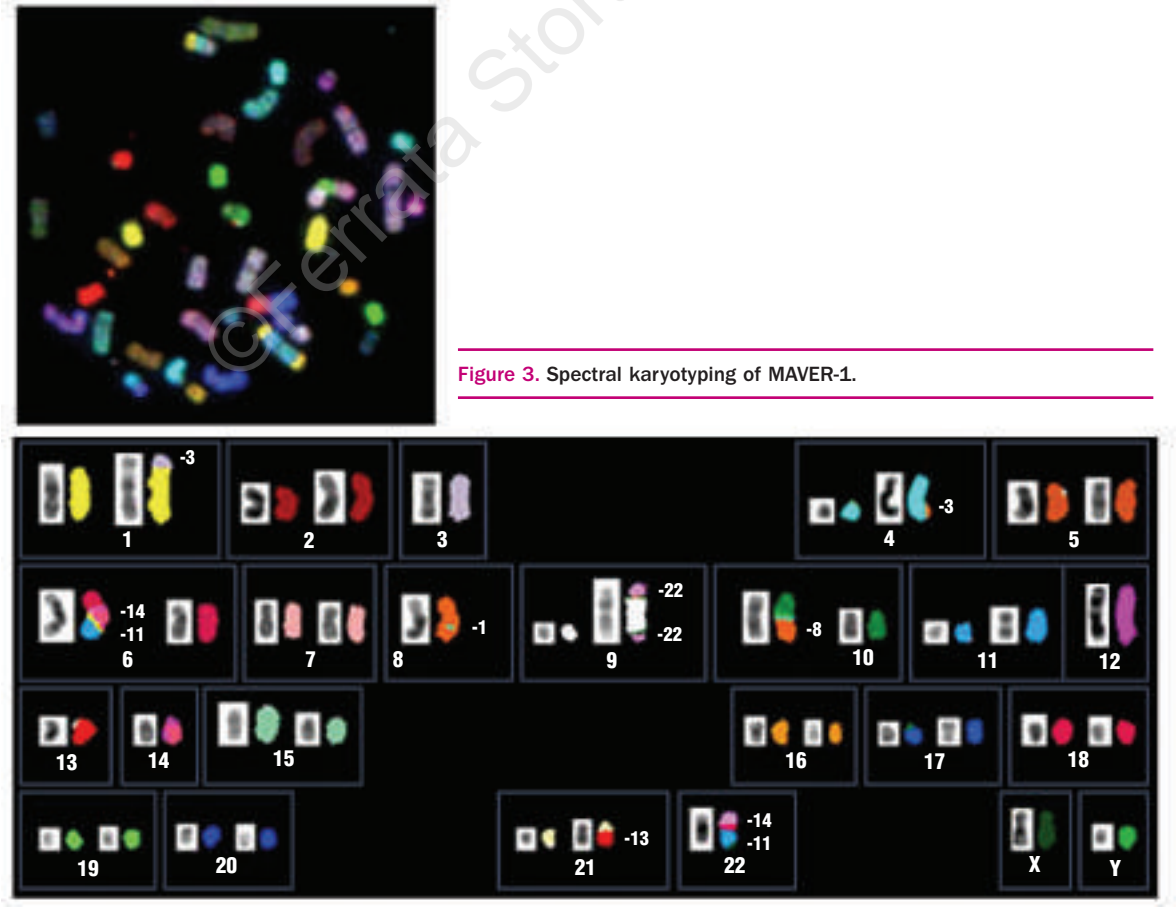

Figure 3. Spectral karyotyping of MAVER-1.

Table 3. FISH results.

Localization	Probe	Cut-off	Result
3q27	LSI BCL6 Dual Color, Abbott GmbH&Co.KG	≥6%	Normal
5q31	LSI EGR1(5q31)/D5S721, D5S23(5p15.2), Abbott GmbH&Co.KG	≥6%	Tetrasomy in 15%
5p15	LSI EGR1(5q31)/D5S721, D5S23(5p15.2), Abbott GmbH&Co.KG	≥6%	Tetrasomy in 14%
6q27	Yac 977e10	≥8%	Deletion in 85%
7p15	Yac 961b12	≥8%	Deletion in 43%
8p22	Yac 948d5	≥9%	Deletion in 82%
8q24	LSI MYC Break-apart, Abbott GmbH&Co.KG	≥9% in 70%	Rearranged in 100%; trisomy
$P16^{INK4a}$	LSI p16(9p21)/CEP9, Abbott GmbH&Co.KG	≥5%	Deletion in 35%
ATM2	PAC LLNLP704001298Q19	≥6%	Deletion in 73%
t(11;14)(q13;q32)	LSI IGH/CCND1, Abbott GmbH&Co.KG	≥9%	t(11;14) in 100%
12q12/13	Yac 743a1	≥6%	Deletion in 92%
D13S25	LSI-D13S25(13q14.3), Abbott GmbH&Co.KG	≥5%	Deletion in 94%
15q23	Yac 954e9	≥10%	Trisomy in 36%
TP53	LSI-p53(17p13.1), Abbott GmbH&Co.KG	≥5%	Deletion in 90%

200 cells derived from five normal cell suspensions (reactive lymph node specimens), and calculating the mean prevalence of a given signal plus or minus three standard deviations.

Spectral karyotyping

Chromosome preparations stored at -80°C were used, and hybridization was performed with a SKY fluorescent labeling kit (Applied Spectral Imaging, Migdal HaEmek, Israel). After hybridization chromosomes were counterstained with DAPI. Images were acquired using a SD200 Spectracube system (ZEISS) with a custom-designed optical filter (SKY-1, Chroma Technology, Brattleboro, VT, USA). Chromosomal material was identified by calculating a false-color picture from the emitted spectra.[23]

Results

Primary lymphoma

Both spleen and lymph node showed effaced architecture with large partially confluent nodules predominantly composed of medium-sized cells, with moderately abundant cytoplasm, a round or slightly irregular nucleus with moderately dispersed chromatin and one or more small nucleoli. Mitoses were relatively uncommon (<10/10 high-power fields). Neoplastic cells were $CD20^+$, $CD5^+$, cyclin $D1^+$, $CD23^-$, $CD10^-$ (Figure 1). p53 was expressed in 30% of the cells.

Cell line morphology and growth

After ethanol fixation and hematoxylin-eosin or May-Grünwald-Giemsa staining, cells showed a spectrum of medium-sized to large cells, with occasional multinucleated or bizarre cells. Cells had a round-ovoid or moderately irregular nucleus, with granular chromatin distribution, one (rarely two) small nucleoli, and a definite rim of amphophilic cytoplasm (Figure 1). Cells grow singly in suspension in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, and can be frozen in a medium composed of 60% RPMI 1640, 30% heat-inactivated fetal calf serum and 10%DMSO, using a freezing container (5100 Cryo 1°C Freezing Container, Nalgene labware), and revived by standard thawing procedures. Cells are optimally maintained at a density between 500,000 and 2,000,000/mL, show a plateau of growth around 2,500,000/mL, and can be split 1:4 every 72 hours.

Cytochemistry

The cells were negative for periodic-acid Schiff, myeloperoxidase, non-specific esterase, acid phosphatase and alkaline phosphatase.

Immunophenotype

Flow cytometry was performed on the original peripheral blood sample and on the cell line after 10 weeks, 6 months, 8 months and 2 years of continuous culture. The results are summarized in Table 1. Most cell markers remained unchanged, with the notable exceptions of CD5 and IgD, both of which became weaker (IgD expression eventually became negative), and Bcl-2 expression which increased slightly. Western blotting and immunohistochemistry confirmed the $CD20^+$, $CD3^-$, $CD5^+$, $BOB1^+$, $vimentin^+$ phenotype.

Cell cycle deregulation

DNA content was analyzed in the initial peripheral blood sample and in the cell line after 6 months of culture. It showed no evident tetraploid or aneuploid peaks, and revealed a high S-phase (diploid S: 36.52%) in the cell line compared to in the peripheral blood sample (diploid S: 2.16%). Immunohistochemistry showed staining for the proliferation marker Op18/Stathmin in nearly 100% of the cells. TP53 was strongly positive in nearly all cells, while $p21^{WAF1}$ was negative. Staining for the Cdk4 inhibitor CDKN2A (p16) was negative. Western blotting confirmed the presence of Op18/Stathmin, as well as the $TP53^+/p21^-$ phenotype.[24]

Mutational analysis of TP53

A shifted band was revealed in exon 8. Sequencing of both strands of cDNA revealed a mutation at codon 281 with a G→C substitution (D→E amino acid change). Sequencing electropherograms showed a single peak at the mutation site, in accordance with a concomitant deletion of the other allele.

Detection of t(11;14) and cyclin D1 overexpression

PCR amplification revealed the presence of a t(11;14), indicative of a translocation involving the major translocation cluster region, confirmed by cloning and sequencing four clones each of both the peripheral blood and MAVER-1 (Figure 2). By immunohistochemistry cells showed strong staining for cyclin D1 (both DCS-6 and SP4 antibodies), which was also confirmed by western blotting.

Karyotype

The consensus karyotype as defined by classical cytogenetics and SKY harbored extensive structural and numerical chromosome aberrations and was: 46, XY, der(1)t(1;3)(p36;q21or23),-3,der(4)t(4;8)(?q35;?q24), der(6)t(6;14;11)(6pter→6q13::14q11→14q32::11q13→11q25::11q23→11qter),der(7)del(7)(p15.1p15.2)del(7)(q32),-8,del(9)(?q10),der(9)t(22;9;22)(22qter→22q11::9q34→9?→9q34::22q11→22q12or13),der(10)t(8;10)(q13;p13),del(11)(q13),-12,der(12)t(12;12)(12?→12q?::12q21→12q13::12p13→12qter),-13,-14,ins(15)(q12q15q22),add(17)(p13), der(21)t(13;21)(q?;q22),-22,+mar(22;14;11), +2~11mar[5]/46, idem, del(4)(q21) [1]/56, idem, idic(4)(q28)[1]/92,idemx2[8]/92,idemx2,del(4)(q21) [1]/138, idemx3 [1]/184, idemx4, idic(4)(q28) [1] (Figure 3).

FISH

The FISH results are summarized in Table 3. The main findings include the presence of the t(11;14) translocation, the presence of an additional *MYC* rearrangement, *TP53* and *ATM* deletions, as well as deletions of 6q27, 8p22, 12q12/13, 13q14.3 in a high percentage of cells. Deletion of $P16^{INK4A}$, 7p15, and 15q23 gains seem to involve subclones.

VH gene usage

Both the original sample and the cell line presented the same V_H3-9/D3-22/J_H4 combination with a naïve mutational status, although presenting minor intraclonal sequence variations.

EBV status

Staining for LMP-1 was completely negative. EBER was positive in rare cells (about 1/5000) grown for about 6 months. These data are indicative of latent infection in a very small subpopulation of the cells, possibly remnants of long-lived EBV-infected lymphocytes already present in the original sample. EBER was repeated after 2 years of continuous culture and was completely negative.

Discussion

We describe the establishment and accurate molecular characterization of a novel MCL cell line, MAVER-1. The availability of continuous cell lines is vital to any field of oncologic research as it provides an unlimited source of homogeneous study material, particularly for rare diseases. Only nine putative MCL cell lines have been established so far, but some were either not fully characterized, or did not show a classical MCL phenotype.[15,25] MAVER-1 shows almost all the hallmarks of MCL, including cyclin D1 expression, PCR and FISH evidence of t(11;14), positivity for B-cell markers, CD23 negativity, and λ monoclonality. The expression of CD5 was not readily evident by immunohistochemistry, but was clearly positive using more sensitive techniques such as flow cytometry and western blotting. Notably, CD5 expression decreased with time, and the same phenomenon also happened for IgD expression, which was brightly positive on the original peripheral blood sample, but later became only weakly present, and eventually turned negative. The analysis of *VH* gene usage confirmed that the cell line is really derived from the patient, excluding any possible contamination. In the case of lymphoid cell lines this investigation removes the need for validation by DNA fingerprinting.[16] *MAVER-1* uses V_H3-9, D_H3-22, and J_H4 and indeed the V_H3 and D_H3 families are the most common in MCL, as is J_H4.[26]

The immunohistochemical expression of TP53 and lack of p21 is indicative of non-functional TP53.[24] Indeed a codon 281 missense mutation in exon 8, causing a D to E amino acid change in the protein, was associated with the deletion of the other allele. The D281E *TP53* mutation has already been described in a number of tumors (*http://p53.free.fr*) and it is probably of oncogenic importance. Cell cycle deregulation is also demonstrated by DNA content analysis, which shows a clear shift towards the G_2-S phase in comparison to cell cycling status in the original blood sample. Tetraploid peaks were not detected by this analysis, although some polyploid/aneuploid clones were detected morphologically and by cytogenetic analysis.

FISH analysis showed the t(11;14) and additional chromosomal aberrations, some of which merit further discussion. Deletion of 17p13.1 together with the *TP53* mutation evidences a classic two-hit mechanism of deregulation of the TP53 cascade. *ATM* deletion, present in MAVER-1, is a frequently reported abnormality in MCL,[20] while 9p21, containing the locus for *CDKN2A*, is not affected. Deletion of $p16^{INK4A}$, present in a significant subset of MAVER-1 cells, is associated with an aggressive subset of MCL.[27,28] The 8q24 translocation involving *MYC*, present in MAVER-1, defines a subgroup of MCL with a dismal outcome, leukemic involvement and blastic morphology.[29-34] 8q24 abnormalities are associated with 8p losses[35] which are indeed present in MAVER-1; interestingly, many MCL cell lines appear to express *MYC*, an interesting feature since high RNA expression of MYC is associated with poor prognosis in MCL.[36] However, MAVER-1 is the only MCL cell line known to date that has been shown to contain a *MYC* rearrangement. The 13q14 deletion present in MAVER-1 is the most common genetic abnormality in MCL, a feature shared with B-cell chronic lymphocytic leukemia, although the underlying mechanism is still poorly understood.[37,38] The 6q27 deletion is also present in MAVER-1; this aberration has been commonly reported in lym-

phomas,[39,40] and possibly involves the *PDCD2* gene.[40] Curiously our cell line appears to bear a deletion rather than an amplification of 12q12-13, a region usually amplified in MCL, probably affecting *CDK4*.[30,34,41]

Both classic cytogenetics and SKY showed a complex karyotype with multiple aberrations. These analyses suggested that the t(11;14) had undergone further rearrangements involving chromosome 6. Other translocations are also present, such as t(1;3)(p36;q21or23), t(4;8)(q35;q24), t(8;10)(q13;p13), t(12;12), t(13;21)(?;q22), and t(22;9;22). These translocations have never been previously reported in lymphomas, but breakpoints involved have been detected in MCL-associated aberrations.[34] For instance in the Granta-519 cell line 1p36 is rearranged to 14q11 and also shows a cryptic inversion of this locus;[42] comparative genomic hybridization studies show that this region is frequently lost in MCL,[43] suggesting a role in the pathogenesis of MCL.

As previously detailed, the 8q24 region containing the *MYC* gene can be rearranged in MCL.[29-34] In MAVER-1 8q24 is rearranged to 4q35, a highly recombinogenic region involved in a genetic syndrome called facio-scapulo-humeral muscular dystrophy (FSHD),[44] in which intriguingly the cyclin D1 mRNA is upregulated, and MYC downregulated.[45] The 6q23 locus involved in the complex t(6;11;14) is also a breakage hotspot in MCL.[34,43] The t(8;10)(q13;p13) involves the *BMI-1* locus at 10p13,[46] a well-known player in MCL pathogenesis;[30,47] the *AF10* gene at 10p13, a leucine-zipper protein with oncogenic potential[48] might also be involved. The 12p13 locus involved in the t(12;12) translocation contains multiple candidate tumor suppressors,[49] the cyclin D2 repressor,[50] and *GAPD*,[51] all involved in lymphomatous transformation.

In conclusion MAVER-1 is a new MCL cell line with many features of aggressive poor-prognosis MCL, including *TP53* mutations, *ATM* deletion, *MYC* rearrangement, 8q deletions and a complex cytogenetic picture. Its features have been described in detail and will form the basis for its use as an experimental model of MCL. Further characterization and comparison to other MCL cell lines and tissue samples should provide insights into the pathogenesis of MCL.

AZ: cell line establishment and following cell culture work, immunohistochemistry, molecular biology, manuscript preparation and revision; GO: experimental design and supervision concerning FISH, SKY and classical cytogenetics, critical revision of the manuscript; TK: FISH and classical cytogenetic analysis, critical revision of the manuscript; PA: SKY analysis, critical revision of the manuscript; CP: cytofluorimetric analysis, critical revision of the manuscript; AS: molecular biology, experimental design and supervision, critical revision of the manuscript; ML: cytology, experimental design and supervision, critical revision of the manuscript; FM: morphological expertice, experimental design and supervision, critical revision of the manuscript; MC: immunohistochemistry, experimental design and supervision, critical revision of the manuscript.

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