MM-1, a c-Myc-binding Protein, Is a Candidate for a Tumor Suppressor in Leukemia/Lymphoma and Tongue Cancer*

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The c-myc oncogene product (c-Myc) is a transcription factor that dimerizes with Max and recognizes the E-box sequence, and it plays key functions in cell proliferation, differentiation, and apoptosis. We previously showed that MM-1 bound to myc box II within the transactivation domain of c-Myc and repressed the E-box-dependent transcriptional activity of c-Myc. Here we report that MM-1 showed features of a tumor suppressor. In an EST database search for cDNAs homologous to MM-1, we found a frequent substitution of amino acid 157 of MM-1, from alanine to arginine (A157R), and the substitution was observed more in tumor cells than in normal cells. A survey of the A157R mutation of MM-1 in 57 cultured cancer cells and 90 tissues from cancer patients showed that the A157R was present in about 50–60% of leukemia/lymphoma cells and in more than 75% of squamous cell carcinoma of tongue cancer. Although both the A157R and the wild-type MM-1 bound to c-Myc, only A157R lost the activities to repress both the E-box-dependent transcriptional activity of c-Myc and the myc/\(ras\) cooperative transforming activity in rat 3Y1 cells. Furthermore, the wild-type MM-1, but not A157R, arrested the growth of 3Y1 cells. The human MM-1 gene was mapped to chromosome 12q12–12q13, where many chromosome abnormalities in cancer cells have been reported. The results suggest that MM-1 is a novel candidate for a tumor suppressor that controls the transcriptional activity of c-Myc.

c-Myc is a transcription factor, and it plays key functions in cell proliferation, differentiation, and apoptosis (for recent reviews, see Refs. 1–6). c-Myc complexed with Max at the C-proximal region recognizes the E-box sequence in the target genes to be transactivated. Although many candidate genes for c-Myc/Max have been reported, the physiological target genes for c-Myc/Max remain poorly understood. Notably, the results of experiments using c-myc-negative (null) Rat-1 cells showed that of the candidate genes for c-Myc-Max, expressions of only a few of the genes were changed in c-Myc-nul cells (6). Since many candidate genes for transactivation have recently been identified by using the microarray method (7, 8), identification of the \textit{bona fide} target genes of c-Myc should be possible. For its versatile functions, c-Myc associates with various factors other than Max (5), including p107 (9, 10), TBP (11, 12), Bin-1 (13), AMY-1 (14), TRRAP (15), PAM (16), \(\alpha\)-tubulin (17), MM-1 (18), and Cdk inhibitor p21 (19), which bind to the N-proximal region of c-Myc, and also YY-1 (20), Miz-1 (21), AP2 (22), Nmi (23), BRCA1 (24), SNF5 (25), CBF-C/\(NF-YC\) (26), cd2 (27), MSSP (28), CDC6 (29), and Orc1 (30), which bind to the C-proximal region. These binding proteins are thought to modulate c-Myc function, and mutation of the proteins or disrupted expression of their genes may lead to cell transformation by c-Myc. Although it has been shown that translocation of c-myc gene to an immunoglobulin heavy chain gene occurs in Burkitt lymphoma (31), other molecular mechanisms leading to cell transformation by c-Myc have not been clarified, but several models have been proposed. In Burkitt lymphoma, point mutations of amino acids within the N-terminal region of c-Myc, especially threonine at amino acid 58 or serine at 62, have been frequently observed. p107, an \(RB\) family tumor suppressor protein, loses its activity to bind to the N-terminal region of c-Myc due to these point mutations, thereby releasing free active c-Myc (9), although a controversial result has been reported (10). Mutations of amino acid 58 and 62 also prolong the stability of c-Myc, leading to deregulated activation of target genes such as cell cycle-regulating genes (32). In familial adenomatous polyposis, mutation of APC, a tumor suppressor, prevents \(\beta\)-catenin from degradation, leading to the accumulation of \(\beta\)-catenin-Tcf/Lef complex on the \(c-myc\) promoter to activate deregulated c-myc expression (33). \(p15\), an inhibitor of Cdk4, is known to be a tumor suppressor, and it has recently been reported that the \(p15\) gene is up-regulated by Miz-1 by binding to the initiator region of the \(p15\) gene. This activity of Miz-1 to activate the \(p15\) gene is abrogated by formation of Miz-1-c-Myc complex that loses DNA binding activity, thereby progressing the cell cycle (34, 35). TRRAP, a protein related to the ATM/phosphatidylinositol 3-kinase family, binds to the N-terminal transactivation region of c-Myc and recruits an hGCN5 that possesses histone acetyltransferase activity (15, 36). This activity of TRRAP may be necessary for both transcription and cell transforming activities of c-Myc. Mutation of Miz-1 or TRRAP in cancer cells, however, has not been reported.

In addition to a transactivation domain, Myc box II in the N-proximal region of c-Myc also contains a transrepression domain, and several genes repressed by c-Myc have been reported (6, 37). Recent data suggest that transrepression function of c-Myc is correlated more strongly with the transforming activity of c-Myc than its transactivation function (5, 6).
identified a novel protein, MM-1, that binds to this repression domain and represses E-box-dependent transcription activity of c-Myc (18). The mechanism by which MM-1 represses c-Myc activity, however, has not been elucidated.

In this study, we found that a point mutation from alanine to arginine at amino acid 157 in MM-1 frequently occurred in cells from lymphoma, leukemia, and tongue cancer and that this mutation abrogated the inhibitory functions of MM-1 to c-Myc. Human MM-1 gene was mapped at chromosome 12q12–12q13, where many chromosome abnormalities in cancer cells have been reported. Thus, MM-1 is a candidate for a tumor suppressor.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—Human HeLa, rat 3Y1, and mouse Balb3T3 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% calf serum.

**Plasmids**—pCMV-F-MM-1A157T or pCMV-F-MM-1A157R was transcribed with pBR322, respectively, as described previously (18). Two days after transfection, whole cell extract was prepared by addition of the Triton X-100-containing solution to the Pica gene kit (Wako Pure Chemicals Co. Ltd., Kyoto, Japan) to the cells. About one-fifth volume of the extract was used for the β-galactosidase assay to normalize the transfection efficiencies, as described previously (18), and the luciferase activity due to the reporter plasmid was determined using a luminometer, Luminocounter Lumat LB 9507 (EG & G Berthold). The same experiments were repeated five times.

**Northern Blotting**—Balb3T3 cells were cultured under low serum conditions (0.2% calf serum) for 48 h to enter the G0 phase of the cell cycle. At various times after addition of serum to the culture, total RNA was extracted from cells by the guanidine-isothiocyanate method. Twenty µg of RNA was then blotted onto a nitrocellulose filter and hybridized with 32P-labeled human MM-1 cDNA, human c-myc cDNA, or GAPDH as a probe under a high stringency conditions.

**Cloning of Human Genomic DNA of the MM-1 Gene**—Human genomic DNA of MM-1 was obtained after screening high-density gridded filters spotted with colonies of the mouse PAC library RPCII developed by K. Osoegawa and P. de Jong at the Roswell Park Cancer Institute with human MM-1 cDNA as a probe, and 6 positive clones were obtained from the Roswell Park Cancer Institute. Since these clones contain an insert of more than 100 kilobases at the BamHI site of pPAC4, one clone, 57-L5, was digested with BamHI and hybridized with a labeled MM-1 cDNA probe, and the hybridized fragments were inserted into the BamHI site of pBluescript SK(+) (39). After the nucleotide sequences of all of the fragments had been determined, they were aligned as a human MM-1 gene (accession number AB055850).

**FISH Mapping of Human MM-1 Gene**—Lymphocytes isolated from human blood were cultured in α-minimal essential medium supplemented with 10% fetal calf serum and phytohemagglutinin at 37°C for 68–72 h and then treated with bromodeoxyuridine (0.18 mg/ml Sigma) for synchronization. The synchronized cells were washed three times with serum-free medium to release the cell block and resuspended at 3°C for 6 h in α-minimal essential medium containing 2.5 µg/ml thymidine (Sigma). The cells were harvested, and slides were made by the standard procedure, including hypotonic treatment. The cells were then fixed and air-dried. A PAC genomic probe was biotinylated with dATP using a Life Technologies, Inc. BioNick labeling kit (15°C for 1 h) (39). The FISH detection was performed according to the previously described procedure (39, 40). FISH signals and DAPI banding pattern were recorded separately by taking photographs, and the assignment of the FISH mapping data with chromosomal bands was achieved by superimposing FISH signals with DAPI-banded chromosomes (40).

**RESULTS**

**Cloning of Human Genomic DNAs of MM-1**—To obtain the genomic DNA of human MM-1, high-density gridded filters spotted with the colonies of the mouse PAC library were hybridized with a human MM-1 cDNA probe, and fragments from the insert DNA of the positive clone were further subcloned. Finally, a BamHI fragment covering the human MM-1 gene was obtained, and all of the nucleotide sequences were determined (accession number AB055850). Compared with the nucleotide sequence of the MM-1 cDNA, the nucleotide sequences 443–1029 were completely matched with those of genomic DNA. The sequence of MM-1 cDNA spanning nucleotides 1–442 was found to be identical to that in human chromosome 14 and almost identical to that of human endogenous retrovirus type K after a database search of the human genome (Fig. 1A). Partial MM-1 cDNA starting at the nucleotide 464 was first isolated in a screening of proteins encoding c-Myc-binding proteins using a human HeLa cDNA library. Since this cDNA did not contain a putative initiation codon ATG, cDNA screening was further carried out using human a placenta cDNA library in Agt10 with this cDNA as a probe, and MM-1 cDNA (accession number D85807) was obtained as previously described (18). MM-1 genomic DNA isolated in this study revealed that a “new MM-1” comprises 154 amino acids that lack the first 13 amino acids of an “old MM-1.” Since differences between the functions or properties of the new MM-1, human endogenous retrovirus type K which was described as MM-1Δ13 in a previous report, and the old MM-1 were not found (18), we use the old MM-1 as MM-1 in this study. Although the reason why an old MM-1 was isolated is not clear at present,
there are at least two possibilities; one is that MM-1 cDNA in
the placenta library was artificially fused to the sequences from
chromosome 14, and the other is that human endogenous ret-
rovirus type K, whose sequence is almost identical to that of
chromosome 14, was transposed to a region upstream of the
bona fide MM-1 sequence. The human MM-1 gene comprises 6
exons spanning over 4 kilobases (Fig. 1A).

FISH analysis was carried out with a human MM-1 gene
probe. The hybridization efficiency under the conditions used
was ~95% for the probe (i.e. 95 of 100 mitotic cells examined
showed signals on one pair of the chromosomes) (Fig. 1B, cen-
ter). Since DAPI banding was used to identify the specific
chromosome, assignment between the signal from the probe
and the long arm of chromosome 12 was obtained (Fig. 1B, left).
The exact position was determined from 10 photos (Fig. 1B,
right). Since there was no additional locus found by FISH
analysis under the conditions used, the MM-1 gene was deter-
mined to be located at the region q12-q13 of human chromo-
some 12. As described under “Discussion,” this region of chro-
mosome 12 has been found to be a hot spot of chromosome
abnormalities in many cancers. Especially, 35 cases of deletion
of this chromosome region in non-Hodkin’s lymphoma have
been reported in the data base of the Cancer Genome Anatomy
Project of NCBI, which is consistent with the results of MM-1
mutation in cancer patients as shown in Table II.

Expression of mm-1 During the Cell Cycle—To know the
physiological relevance of MM-1 to c-Myc, expression of MM-1
mRNA during the cell cycle was examined. To do this, mouse
Balb/3T3 cells were synchronized to the G0 phase by serum
starvation for 48 h, and the cells reentered G1, S, G2, and M
phases of the cell cycle by the addition of serum. Synchroniza-
tion of cells was first monitored by flow cytometry as described
previously (41). Total RNA was extracted from cells at various
times after addition of serum to the cell culture, and Northern
blotting analysis using these RNAs was carried out with MM-1,
c-Myc, or GAPDH cDNA as a probe (Fig. 2A). Relative expres-
sion levels of mRNAs of c-Myc (c-myc) and MM-1 (mm-1) were
quantitated by normalization of the intensity to that of GAPDH
mRNA (the expression level in cells at random culture being set
to 1) (Fig. 2B). The c-myc was strongly expressed in the early
G1 phase of the cell cycle (4 h), and then decreased. The ex-
pression of mm-1, like that of c-myc, was also strong in the
Balb/3T3 cells were synchronized in the G0 phase of the cell cycle by 15 h later, serum was removed for synchronization in the G0 phase of mRNA. The expression level of the sample from random culture was set to mRNA are quantified and standardized by the amounts of GAPDH myc, as using MM-1, c-

medium and blotted onto filters, and Northern analysis was carried out time when the new medium was added was set to 0. RNAs were cell cycle after the addition of a new medium with 10% serum, and the serum starvation for 48 h. The cells started to enter the S phase of the cell cycle. After 48 h, the cells started to enter the S phase of the cell cycle as in

medium. A one-third of stomach cancer and epidermoid carcinoma cells also possessed A157R of MM-1. Threonine mutation was observed in bile duct carcinoma cells, and no mutations were observed in normal cells. We further screened a total of 88 DNAs from biopsy samples of cancer patients and 25 DNAs from peripheral blood or hair of healthy Japanese in their mid-twenties for MM-1 mutation. Of 39 lymphoma samples, 38% of samples possessed A157R. Especially, 54% of non-Hodgkin’s lymphoma, including 33% of MALT lymphoma, possessed A157R of MM-1 (Table II). Furthermore, squamous cell carcinoma of tongue contained a very high frequency of A157R (72.7%). Only 2 healthy persons carried A157R, while the other 23 had wild-type MM-1. The results implied that normal cells mostly contain wild-type MM-1 and that some types of transformed cells or tumor tissues often carry the Arg mutation at amino acid 157. Since the mutation profile of MM-1 was thus reminiscent of that of p53, we examined the effect of A157R, or A157T, on the biological function of MM-1.

Abrogation of Inhibitory Activities of MM-1 to c-Myc by Arginine Mutation at Amino Acid 157 of MM-1—We have reported that MM-1 is mainly localized in the cell nucleus and is bound to the myc box II in the transcription domain of c-Myc (18). The localization and the binding activity to c-Myc were hence compared between the wild-type and the mutants of MM-1. The A157T or A157R of MM-1, as well as wild-type MM-1, was expressed in E. coli or the synthetic peptide corresponding to the hydrophobic region of MM-1, none of the antibodies recognized the endogenous MM-1 in cells, including antibodies against immunogen of the recombinant MM-1 expressed in E. coli or the synthetic peptide corresponding to the hydrophobic region of MM-1, none of the antibodies recognized the endogenous MM-1 in several cells. We therefore used the ectopic expression experiment of MM-1 in cells. Mouse Balb/3T3 cells were transfected with expression vector for Flag-tagged MM-1 and synchronized to the G0 phase by serum starvation as above. Total protein was extracted from cells at various times after addition of serum to the cell culture, and Western blotting analysis was carried out with an anti-Flag, an anti-c-Myc or an anti-actin antibody (Fig. 2C). The c-Myc was strongly expressed after 4 h, peaked at 8 h, and then decreased. The expression of Flag-MM-1 was also strong at 4 and 8 h, then decreased, and re-expressed 24 h after the addition of serum. These results suggest that MM-1 is coordinately expressed during G1 and S phases with c-Myc and that MM-1 during G2 and M phases functions independently to c-Myc.

Mutation at Amino Acid 157 of MM-1 in Cancer Cells—An EST database search for cDNAs encoding proteins homologous to MM-1 was carried out. Compared with the MM-1 cDNA that we cloned from a human HeLa cell cDNA library, 68 of the 172 homologues carried variations of the amino acid sequence. In this paper, we tentatively call the MM-1 cloned from HeLa cells wild-type MM-1. Substitutions of amino acids were observed over the entire region, but a frequent mutation at amino acid 157 was evident. The mutation at 157, from Ala to either Thr or Arg (A157T or A157R, respectively), corresponding to the nucleotide sequence from GCC to ACC or CGC, was found more frequently in tumor cells than in normal cells in all cases. Furthermore, the difference between normal and tumor cells was most obvious in the frequency of A157R. The percentage of A157R in tumor cells was 10 times higher than that in normal cells. In normal cells, most of the substitution was A157T (data not shown). To see the mutations of MM-1 in the cells, DNAs from 54 cultured human cells of normal and tumor cell origin were extracted, and the fragment corresponding to nucleotide numbers 824–919 of MM-1 cDNA was amplified by using various DNAs as templates, or RT-PCR on the RNAs extracted from human HL60, K562, and Jurkat cells of cancer origin was performed. Sequencing analysis of the fragments showed that 7 of 13 leukemia cell lines, including B and T cell leukemia, all three squamous cell carcinoma cell lines of tongue, and both thyroid adenocarcinoma cells possessed A157R (Table I). One-third of stomach cancer and epidermoid carcinoma cells also possessed A157R of MM-1. Threonine mutation was observed in bile duct carcinoma cells, and no mutations were observed in normal cells. We further screened a total of 88 DNAs from biopsy samples of cancer patients and 25 DNAs from peripheral blood or hair of healthy Japanese in their mid-twenties for MM-1 mutation. Of 39 lymphoma samples, 38% of samples possessed A157R. Especially, 54% of non-Hodgkin’s lymphoma, including 33% of MALT lymphoma, possessed A157R of MM-1 (Table II). Furthermore, squamous cell carcinoma of tongue contained a very high frequency of A157R (72.7%). Only 2 healthy persons carried A157R, while the other 23 had wild-type MM-1. The results implied that normal cells mostly contain wild-type MM-1 and that some types of transformed cells or tumor tissues often carry the Arg mutation at amino acid 157. Since the mutation profile of MM-1 was thus reminiscent of that of p53, we examined the effect of A157R, or A157T, on the biological function of MM-1.
were transfected into human HeLa cells, and the proteins in the cells were detected with an anti-FLAG antibody and fluorescein isothiocyanate-conjugated anti-mouse IgG (Fig. 3B).

TABLE I
Mutation states of MM-1 in cancer cells and in cells of normal origin

| Cell line    | Characteristics            | MM-1 mutation |
|--------------|----------------------------|---------------|
| Jurkat       | Acute T cell leukemia      | R             |
| HPB-ALL      | T cell leukemia            | R             |
| SKW-3        | T cell leukemia            | wt            |
| PELL         | Acute T cell lymphocytic leukemia | wt      |
| HL-60        | Acute promyelocytic leukemia | R             |
| BALL-1       | B cell leukemia            | R             |
| K-562        | Chronic myelogenous leukemia | R             |
| THP-1        | Monocytic leukemia         | wt            |
| KG-1a        | Erythroblastemia            | wt            |
| Ku812-F      | Basophilic leukemia        | R             |
| MOLT-4       | Acute lymphoblastic leukemia | wt           |
| P30/OHK      | Acute lymphoblastic leukemia | R             |
| EoL-3        | Eosinophilic leukemia      | wt            |
| Raji         | Burkitt’s lymphoma         | wt            |
| Daudi        | Burkitt’s lymphoma         | wt            |
| U-937        | Histiocytic monocyte lymphoma | wt         |
| HPB-MLT      | T lymphoma                 |               |
| SAS          | Tongue squamous cell sarcoma | R             |
| HSC-3        | Tongue squamous cell carcinoma | R         |
| SCC4         | Tongue squamous cell carcinoma | R         |
| 8505C        | Thyroid adenocarcinoma     | R             |
| 8305C        | Thyroid adenocarcinoma     | R             |
| NUGC-4       | Stomach signet ring cell carcinoma | R |
| AZ-521       | Stomach gastric cancer     | wt            |
| SH-10-TC     | Stomach cancer             | wt            |
| HEp-2        | Epidermoid carcinoma       | R             |
| ME-180       | Epidermoid carcinoma       | wt            |
| A-431        | Epidermoid carcinoma       | wt            |
| Ovkl18#102   | Ovarial carcinoma          | wt            |
| Ishikawa     | Ovarial carcinoma          | wt            |
| C33A         | Ovarial carcinoma          | wt            |
| SiHa         | Ovarial squamous carcinoma | wt            |
| 143Btk       | Osteosarcoma               | wt            |
| MG-63        | Osteosarcoma               | wt            |
| Saos-2       | Osteosarcoma               | wt            |
| COLO 205     | Colon, adenocarcinoma      | wt            |
| SW490        | Colon, adenocarcinoma      | wt            |
| A549         | Lung carcinoma             | wt            |
| Lu99         | Lung carcinoma             | wt            |
| HSC-2        | Mouth squamous cell carcinoma | wt         |
| HuCCT1       | Bile duct carcinoma        | T             |
| VMRC-RCW     | Renal cell carcinoma       | wt            |
| MCF7         | Breast adenocarcinoma      | wt            |
| EJ-1         | Urinary bladder carcinoma  | wt            |
| PK-8         | Pancreas carcinoma         | wt            |
| SCC          | Rectal carcinoma           | wt            |
| HT1080       | Fibrosarcoma               | wt            |
| IMR-32       | Neuroblastoma              | wt            |
| Terato       | Teratocarcinoma            | wt            |
| SK-MEL-28    | Malignant melanoma         | wt            |
| HuH-28       | Cholangiocellular carcinoma | wt         |
| Hep G-2      | Hepatoblastoma             | wt            |
| SV80         | SV40 transformed fibroblast | wt         |
| TAKAKO-SV    | SV40 transformed normal skin | wt       |
| 1077-SV      | SV40 transformed normal skin | wt         |
| WI-38        | Normal embryonic lung      | wt            |
| TL-Mor       | Normal lymphocytes         |               |

Both the mutants A157T and A157R were observed in the cell nucleus as well as wild-type MM-1.

Wild-type MM-1 has been shown to repress the E-box-de-
pendent transcription activity of c-Myc in monkey CV-1 cells (18). The effect of the mutation at amino acid 157 on the transcriptional activity was therefore examined. The expression vector for the wild-type, A157T, or A157R of MM-1, or the vector alone, was transfected to human HeLa cells together with a c-myc expression vector and a reporter 4xE-box-SVP-Luc. Forty-eight hours after transfection, the luciferase activity was examined (Fig. 4). It was found that wild-type MM-1 repressed the E-box-dependent transcription activated by c-Myc in HeLa cells, as reported previously (18). The A157T showed a repression activity similar to, or even stronger than, that of wild-type MM-1. The A157R, on the other hand, completely lost its repression activity. The results suggested that the substitution of amino acid 157 of MM-1 from Ala to Arg abrogated the repression activity toward c-Myc/E-box transcription.

We then examined whether the promotion by MM-1 of the transforming activity of c-Myc was also affected by a specific amino acid substitution. Rat normal diploid 3Y1 cells, which have been used for testing the transforming function of various viral and cellular oncogenes (42), were transfected with various combinations of c-myc, activated H-ras, and the wild-type (wt) or mutated (A157T or A157R) mm-1. Fourteen days after transfection, the transformed cell foci were counted (Fig. 5). The c-myc/H-ras cointroduction produced numerous foci, while either c-myc or H-ras alone

TABLE II

| Type of cancer      | Total number | No. of A157R | Ratio |
|--------------------|--------------|--------------|-------|
| Lymphoma           | 39           | 15           | 38%   |
| Tongue cancer (SCC)| 11           | 8            | 72.7% |
| Stomach cancer     | 2            | 0            | 0     |
| Colon cancer       | 8            | 0            | 0     |
| Lung cancer        | 14           | 0            | 0     |
| Breast cancer      | 4            | 0            | 0     |
| Pancreas cancer    | 3            | 0            | 0     |
| Testis cancer      | 1            | 0            | 0     |
| Ren cancer         | 3            | 0            | 0     |
| Liver cancer       | 2            | 0            | 0     |
| Liposarcoma        | 1            | 0            | 0     |
| Normal blood       | 10           | 1            | 10%   |
| Normal hair        | 15           | 1            | 6.7%  |
with c-myc, hand, did not reduce the activity of the A157T mutant of cooperative transforming activity was also observed when G418-resistant cell colonies were counted. Fourteen days after transfection, the mutants (A157T and A157R), and they were cultured in the presence of G418. 

The results suggested that the wild-type and the A157T of MM-1 arrested cell growth but that the A157R mutant lost its arresting activity and even promoted cell growth, probably by antagonizing the activity of endogenous MM-1.

The results indicated that MM-1 suppressed the transforming activity of c-Myc and that the point mutation from Ala to Arg at amino acid 157 of MM-1, which was frequently observed in tumor cells, abrogated the suppression activity.

Tumor suppressor such as Rb family proteins is known not only to suppress tumor formation but also to arrest cell growth. We therefore examined MM-1 for its cell growth arresting activity. Rat 3Y1 cells were transfected with an expression vector for wild-type MM-1, A157T, A157R, or p107 as a control. All of the plasmids used contained the neomycin-resistant gene. The transfected cells were cultured in the presence of G418 for 14 days, and the number of G418-resistant colonies was counted. As shown in Fig. 6B, ~120 colonies were yielded among the cells transfected with the vector alone. The number of G418-resistant colonies was reduced by 25% by additional expression of p107, an Rb family protein binding to the transactivating domain of c-Myc. In the same system, the introduction of wild-type MM-1 reduced the number of colonies to less than half (~40%) of that without MM-1. The expression of A157T also reduced the number of colonies to a similar level (~50%). The other mutant, A157R, on the other hand, increased the number of colonies to 160% of that without MM-1. The results suggested that the wild-type and the A157T of MM-1 arrested cell growth but that the A157R mutant lost its arresting activity and even promoted cell growth, probably by antagonizing the activity of endogenous MM-1.

**DISCUSSION**

In this study, we first determined the genomic structure and chromosome location of the human MM-1 gene that codes for MM-1, a negative regulator of c-Myc, and then revealed that MM-1 is a candidate for a tumor suppressor in leukemia, lymphoma, and tongue cancer. As described under “Results,” about one-third of the nucleotide sequence from the 5'-end of MM-1 cDNA that we have reported was found to be derived from sequences of chromosome 14 or human endogenous retrovirus type K, thereby leading to the addition of 13 amino acids to the N terminus of MM-1 originating from chromosome 12. Since the results described in our previous report (18) and the results of the current experiments showed no difference between the properties of the fused-type MM-1 and MM-1, we used the fused-type MM-1 throughout this study. In the present study, we also identified at least 4 alternative splicing variants of MM-1 (MM-1α, MM-1β, MM-1γ, and MM-1δ), and MM-1 originating from chromosome 12 was renamed MM-1α. Characterization of these variants will be described elsewhere.

MM-1 was found to be expressed coordinately with c-Myc during G1 and S phases of the cell cycle and re-expressed after the G2 phase, which might affect the functions of MM-1 that are dependent on and independent of c-Myc. MM-1 is located mainly in the cell nucleus, where c-Myc is co-localized (18). It has been reported, on the other hand, that MM-1 is a subunit of prefoldin/Gim, a new chaperon protein complex sorting unfolded proteins to a chaperonin in which unfolded proteins are folded (43, 44). Prefoldin/Gim is composed of six subunits, including putative transcription factors and a VHL-binding protein, and MM-1 has been identified as prefoldin 5/Gim 5 (43, 44). MM-1 in prefoldin/Gim is therefore thought to function independently of c-Myc.

A substitution from Ala to Arg at amino acid 157 in MM-1 was frequently observed in tissue culture cells and cells from patients with leukemia, lymphoma, and tongue cancer. Since other genomic DNAs possessing very similar sequences to that of the MM-1 gene in chromosome 12 exist in several chromosomes such as 11, 7, and X, and these prevent specific primers from being set for the direct sequencing by PCR on genomic DNA as template, the DNA fragments including this mutation site were first amplified by PCR using the genomic DNA or total RNAs as templates and then cloned into plasmid vectors for sequencing. We usually sequenced more than three clones in one sample and classified MM-1 as an Arg mutant when at least a clone contained an Arg substitution. This analysis did not enable precise determination of whether the Arg mutation is homozygous or heterozygous. Furthermore, to determine whether this mutation is hereditary or sporadic, MM-1 genes from a family of lymphoma patient were analyzed, and no mutations were found in this family members. We are currently carrying out linkage analysis using many more samples to try to clarify the above possibilities regarding the MM-1
gene. FISH analysis showed that the MM-1 gene is located at chromosome 12q12–12q13. A search of the data base of the Cancer Genome Anatomy Project of NCBI revealed that this region has been reported to be a hot spot of chromosome abnormalities in cancer cells, including inversion, translocation, and deletion in non-Hodgkin’s lymphoma, liposarcoma, heman-giopericytoma, lipoma, clear cell sarcoma, germ cell tumor, acute myeloid leukemia, or adenocarcinoma.

MM-1, a protein associated with c-Myc at myc box II in the transactivation domain, repressed the E-box-dependent transactivation by c-Myc via control of the transcriptional activity of c-Myc (18) and also the myelosin cooperative cell transforming activity. The cell growth was arrested when the expression vector for MM-1 was introduced. MM-1 was thought to induce cell transforming activity, which suppresses cell progression by c-Myc via control of the transcriptional activity of c-Myc. Moreover, these activities of MM-1 as a tumor suppressor were abrogated by a point mutation at amino acid 157 of MM-1 from Ala to Arg, frequently found in cells of leukemia, lymphoma, and carcinoma of the tongue. Cells may have more chances to be transformed when MM-1 carries an A157R-like mutation and has lost its tumor suppressing activity.

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