MDM2 Protein Overexpression Promotes Proliferation and Survival of Multiple Myeloma Cells

By Gerrard Teoh, Mitsuyoshi Urashima, Atsushi Ogata, Dharminder Chauhan, James A. DeCaprio, Steven P. Treon, Robert L. Schlosman, and Kenneth C. Anderson

The murine double minute 2 (MDM2) protein facilitates G1 to S phase transition by activation of E2F-1 and can enhance cell survival by suppressing wild-type p53 (wtp53) function. In this study, we examined MDM2 expression and function in multiple myeloma (MM) cells. MDM2 is strongly and constitutively expressed in MM cell lines (ARH-77, RPMI 8226, and OCI-My5) and in the cells of plasma cell leukemia (PCL) patients, but is not expressed in normal bone marrow mononuclear cells (BM MNCs). Treatment of MM cells with MDM2 antisense, but not sense, nonsense, or scrambled, oligodeoxyribonucleotides (ODNs) decreased DNA synthesis and cell viability; it also induced G1 growth arrest, as evidenced by propidium iodide (PI) staining and induction of retinoblastoma protein (pRB) to E2F-1 binding. Moreover, inhibition of MDM2 using antisense ODNs also triggered MM cell apoptosis as evidenced by acridine orange–ethidium bromide staining. We next studied the association of MDM2 with wtp53 and/or mutant p53 (mtp53), E2F-1, CDK4, and p21. MDM2 constitutively binds to E2F-1 in all MM cells, to both wtp53 and mtp53, and to p21 in tumor cells lacking p53. These data suggest that MDM2 may enhance cell-cycle progression in MM cells both by activating E2F-1 and by downregulating cell-cycle inhibitory proteins (wtp53 and p21). Overexpression of MDM2 may therefore contribute to both growth and survival of MM cells, suggesting the potential utility of treatment strategies targeting MDM2 in MM.

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MM cell lines and PCL patient samples. The human MM-derived cell lines used were ARH-77 (American Type Culture Collection [ATCC] CRL-1621, Rockville, MD), RPMI 8226 (ATCC CCL-155), U266 (ATCC TIB-196), and OCI-My5 (a gift from Dr H.A. Messner, Ontario Cancer Institute, Toronto, Canada). ARH-77 and RPMI-8226 MM cells were cultured in RPMI 1640 with 1-glutamine medium (Cellgro; GIBCO-BRL, Gaithersburg, MD) supplemented with 10% heat-treated fetal bovine serum (FBS) (PAA Laboratories Inc, Newport Beach, CA), 25 U/mL penicillin and 25 mg/mL streptomycin (Pen-Strep; GIBCO-BRL), and 5 mMol/L 1-glutamine (GIBCO-BRL). U266 MM cells were cultured in RPMI-1640 with 1-glutamine medium supplemented with 15% FBS, Pen-Strep, and 5 mMol/L 1-glutamine. OCI-My5 MM cells were cultured in Iscove’s modified Dulbecco’s medium (Sigma Diagnostics, St Louis, MO) supplemented with 10% FBS, Pen-Strep, and 5 mMol/L 1-glutamine. Saos-2 (ATCC HTB-85), a human osteosarcoma cell line without detectable p53 protein, was cultured in McCoy’s 5A (modified) medium with 1-glutamine (GIBCO-BRL) supplemented with 15% FBS, Pen-Strep, and 5 mMol/L 1-glutamine. All cell lines were grown at 37°C in a humidified 5% CO2 atmosphere.

Mononuclear cells (MNCs) were obtained by Ficoll-Paque (Pharmacia Biotech, Uppsala, Sweden) separation from peripheral blood samples of two patients with PCL (PCL1 and PCL2). The MNC fraction in both PCL1 and PCL2 included more than 95% CD38+CD45RA+ cells. The PCL1 and PCL2 fractions in both PCL1 and PCL2 included more than 95% CD38+CD45RA+ cells.

Antibodies. The following primary monoclonal antibodies (MoAbs) were used: SMP14 anti-MDM2 MoAb recognizing amino acid residues 154 to 167 of human MDM2, DO-1 horseradish peroxidase (hrp)-conjugated anti-p53 MoAb recognizing pancept p53 (ptp53), and C-20 anti–E2F-1 MoAb (all Santa Cruz Biotechnology Inc). HRP-conjugated sheep anti-mouse Ig MoAb and C-20 anti–E2F-1 MoAb (both Santa Cruz Biotechnology Inc). HRP-conjugated sheep anti-mouse Ig MoAb and C-20 anti–E2F-1 MoAb (both Amersham Life Science, Buckinghamshire, UK) were used as secondary MoAbs.

Cell lines were grown to 70% to 80% confluency, harvested, and pelleted by centrifugation at 3,000 g for 5 minutes at room temperature. Each cell pellet was washed thrice with 50 mL phosphate-buffered saline (PBS) (Sigma Diagnostics) for MM cell lines or Tris-buffered saline (TBS) for PCL cells, and then lysed on ice for 30 minutes in lysis buffer (containing 50 mMol/L Tris, pH 8.0, 150 mMol/L NaCl, 0.1% Nonidet P-40, 1.0 mMol/L EDTA, 50 mMol/L sodium fluoride, 1.0 mMol/L sodium orthovanadate, 2.0 g/mL aprotinin, 2.0 g/mL leupeptin, and 5.0 g/mL phenylmethylsulfonyl fluoride) with frequent vortexing. Cell lysates were centrifuged at 6,000 × g for 15 minutes at 4°C, and the supernatants were divided into 1 mL aliquots and transferred to 1.5 mL Eppendorf tubes. Each aliquot was preclared by incubating with 1 mL rabbit anti-mouse ascites fluid and 100 g 10% (vol/vol) Protein-A Sepharose CL-4B beads (Pharmacia Biotech) for 1 hour at 4°C with continuous rocking. The Sepharose beads were then precipitated by centrifugation at 10,000 g for 30 seconds, and the supernatants were collected and assayed for total protein content by Bradford’s microtiter plate method (Biorad, Hercules, CA).

For Western blotting, 100 μg whole cell lysate was equally loaded into each well of a sodium dodecyl sulfate (SDS)-polyacrylamide gel. For immunoprecipitation, 500 μg whole cell lysate was first incubated with 1.0 μg of the antibody of interest for 1 hour at 4°C with continuous rocking. Thereafter, 100 μL 10% (vol/vol) Protein-A Sepharose CL-4B beads were added and incubated overnight at 4°C with continuous rocking. The Sepharose beads were precipitated the next day by centrifugation at 10,000 g for 30 seconds and the supernatant was discarded. The beads were washed thrice with lysis buffer and pelleted; the final supernatant was discarded. The remaining lysis buffer was removed by aspiration, and the pellet was resuspended in 25 μL 1 × sample buffer (containing 2% SDS, 60 mMol/L Tris, pH 6.8, 10% 2-mercaptoethanol, 20% glycerol, and 0.001% bromophenol blue). After a brief vortex mixing, the sample was incubated in a water bath at 85°C for 10 minutes, and then vortexed for 30 seconds and centrifuged at 16,000 g for 15 minutes. Twenty-five microilters of the immunoprecipitated sample was equally loaded into each well of 6% (pRB), 8% (MDM2, E2F-1, and p53), 10% (CDK4), and 12% (p21) SDS-polyacrylamide gels.

After both whole cell lysates and immunoprecipitated samples were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE), proteins were transferred onto polyvinylidene difluoride (PVDF) membranes (Immobilon-P, Millipore, Bedford, MA) by a semidry method. The membranes were next blocked by incubating overnight at room temperature with continuous rocking in blotto (containing 5% skim milk [Mix & Drink; Saco Foods Inc, Middleton, WI], 0.2% Tween-20 [Sigma Diagnostics], and 0.02% sodium azide) and then washed in Tris-buffered saline with Tween-20 (TBST) (containing 20 mMol/L Tris, pH 7.6, 150 mMol/L NaCl, and 0.05% Tween-20) for 45 minutes with continuous rocking and frequent changes of TBST. Membranes were next incubated for 1 hour at room temperature with the appropriate primary Abs diluted in TBST to a final concentration of 1 μg/mL. Washing was then performed as already described, and when required, membranes were incubated for 45 minutes at room temperature with secondary hrp-conjugated antibodies diluted 1:1,000 in TBST. After the final wash, the hrp chemiluminescence detection was performed as directed by the manufacturer (ECL, Amersham Life Science), followed by exposure to photographic film (BioMax MR; Eastman Kodak Co, Rochester, NY) for 5 to 60 seconds.

MDM2 antisense, sense, and nonsense ODN assays. RPMI 8226 MM cells were cultured in 96-well tissue culture plates at an initial cell density of 1.7 × 104 cells/mL for cell proliferation and cell density analyses, and in 24-well tissue culture plates at an initial cell density of 0.5 × 106/mL for cell-cycle and apoptosis analyses. Twenty-molar HEPES buffer (Intergen, Purchase, NY) was added to wells receiving ODNs; control cells were cultured in media without addition of ODNs. The MDM2 ODN sequences were as follows: 15-base antisense ODN (A15) (5′-dGACATGTTGATTTG-3′); 20-base antisense ODN (A20) (5′-dGACATGTTGATTTGACAT-3′); 15-base sense ODN (S15) (5′-dATGCACTACAAAC-3′); 20-base sense ODN (S20) (5′-dATGCACTACAAACATGTC-3′); 15-base nonsense ODN (N15) (5′-dATGCACTACAAACATGTC-3′); 20-base nonsense ODN (N20) (5′-dATGCACTACAAACATGTC-3′); 15-base scrambled ODN (X15) (5′-dATGCACTACAAACATGTC-3′); and 20-base scrambled ODN (X20) (5′-dATGCACTACAAACATGTC-3′). ODNs were used at a concentration of 0.1, 0.01, and 2.0 μmol/L to assay for effects on the kinetics of proliferation; a cell survival profile, apoptosis assay, and immunoblotting were performed in cells cultured with ODNs at 1.0 μmol/L.

For cell proliferation analyses, cells were cultured for periods of
up to 24 hours with each ODN (antisense [A15 and A20], sense [S15 and S20], nonsense [N15 and N20], and scrambled [X15 and X20]), and DNA synthesis was assayed by tritiated (H) thymidine (TdR) uptake. In some cultures, ODNs were replenished every 4 hours. Briefly, 0.5 μCi 3H-TdR (Dupont NEN, Boston, MA) was added to each culture well and incubated for 4 hours before harvesting onto glass filters using the HARVESTER 96 MACH II (Tomtec Inc, Orange, CT) and counting on the 1205 BETAPLATE liquid scintillation counter (Wallac, Gaithersburg, MD). Cell density was simultaneously determined from an aliquot (10 μL) of each well by trypan blue (GIBCO-BRL) exclusion.

For cell-cycle and apoptosis analyses, cells were similarly cultured for periods of up to 24 hours with ODN, washed thrice in ice-cold PBS, and pelleted. The cell pellet was resuspended in 0.5 mL ice-cold propidium iodide (PI) 15 μg/mL; Sigma Diagnostics) in 0.1% sodium citrate and 0.1% Nonidet P-40, and incubated for 30 minutes at 4°C. The cell-cycle distribution of these cells was analyzed by flow cytometry (Coulter Corp, Miami, FL). The percentage of apoptotic cells was determined simultaneously from an aliquot (10 μL) of each well obtained before PI staining by acridine orange (100 μg/mL) and ethidium bromide (100 μg/mL) (both Sigma Diagnostics) staining. Apoptotic cells were enumerated by fluorescence microscopy (Microstar IV; Reichert-Jung, Buffalo, NY) at 490 nm excitation wavelength; the mean percentage of apoptotic cells was determined for three samples (200 cells per sample).

**RESULTS**

Expression of MDM2 protein in MM-derived cell lines, cells from PCL patients, Saos-2 osteosarcoma cells, and normal BM MNCs. MDM2 protein expression in MM cell lines and cells from PCL patients was compared with the expression in p53-deficient Saos-2 osteosarcoma cells and normal BM MNCs. Figure 1 shows that MM-derived cell lines and PCL cells, but not normal BM MNCs, constitutively express MDM2 protein. Saos-2 osteosarcoma cells, which strongly express MDM2, served as positive controls. In addition, we also examined γ-irradiated BM MNCs, since x-ray therapy (XRT) has been reported to increase MDM2 expression. However, we were unable to increase MDM2 expression in normal BM MNCs. Immunoblotting with anti-actin MoAb confirms equal protein loading.

Effect of culture with MDM2 antisense ODN on expression of MDM2, p53, E2F-1, CDK4, and p21 in RPMI 8226 MM cells. To assess the function of MDM2 antisense ODN on proliferation of RPMI 8226 MM cells, we next attempted to block MDM2 expression using specific antisense ODN (A20). Figure 2 shows that MDM2 protein expression in RPMI 8226 cells was inhibited in cells cultured with MDM2 antisense ODN, but not in cultures with media, MDM2 sense ODN (S20), or MDM2 nonsense ODN (N20). In contrast, no significant changes were evident in the constitutive expression of p53, E2F-1, CDK4, and p21 in RPMI 8226 cells treated with MDM2 antisense ODN compared with cells cultured with media, MDM2 sense ODN, or MDM2 nonsense ODN, confirming the specificity of MDM2 antisense ODN action.

Half-life of MDM2 in RPMI 8226 MM cells. Three previous reports have indicated that MDM2 has a half-life of 15 to 30 minutes in rat embryo fibroblasts, 3T3DM cells, and choriocarcinoma cells (JAR and JEG-3). We next determined the half-life of MDM2 in RPMI 8226 MM cells using 35S-methionine pulse-chase experiments. Figure 3 shows that MDM2 expression is markedly reduced at 30 minutes, suggesting that the half-life of MDM2 in RPMI 8226 cells is approximately 25 minutes.

Effect of culture with MDM2 antisense ODN on proliferation in RPMI 8226 MM cells. To determine the kinetics of X20 ODN cultures, tumor cells x-ray therapy (XRT) has been reported to increase MDM2 expression which strongly express MDM2, served as positive controls. In addition, we also examined γ-irradiated BM MNCs, since x-ray therapy (XRT) has been reported to increase MDM2 expression. However, we were unable to increase MDM2 expression in normal BM MNCs. Immunoblotting with anti-actin MoAb confirms equal protein loading.

Effect of culture with MDM2 antisense ODN on cell-cycle distribution and apoptosis in RPMI 8226 MM cells. Having established the inhibitory effect of MDM2 antisense ODN on proliferation of RPMI 8226 MM cells, we next assayed the effects of MDM2 antisense ODN on cell-cycle distribution and apoptosis. RPMI 8226 cells were cultured with MDM2 antisense ODN (A15), media alone, MDM2 sense ODN (S15), or MDM2 scrambled ODN (X15) at concentrations of 1.0 μmol/L for up to 24 hours, without replen-
Fig 1. Expression of MDM2 protein in MM-derived cell lines, PCL patients cells, Saos-2 osteosarcoma cells, and normal BM MNCs. Total cell lysates of MM cell lines (ARH-77, RPMI 8226, and OCI-My5), cells from PCL patients (PCL1 and PCL2), Saos-2 p53-deficient osteosarcoma cells, and normal BM MNCs (without and 1 and 4 hours post-XRT) were immunoprecipitated with SMP14 anti-MDM2 MoAb followed by immunoblotting with the same Ab. Immunoprecipitation and immunoblotting with AB-1 anti-actin MoAb confirmed equal protein loading.

MDM2 inhibits p53-mediated apoptosis, we next tested for wtp53 and mtp53 expression and p53 binding to MDM2 in MM cell lines, cells from PCL patients, Saos-2 p53-deficient osteosarcoma cells, and normal BM MNCs. Since MDM2 inhibits p53-mediated apoptosis, we next tested for wtp53 and mtp53 expression and p53 binding to MDM2 in MM cell lines, cells from PCL patients, Saos-2 p53-deficient osteosarcoma cells, and normal BM MNCs. We also examined γ-irradiated BM MNCs, since XRT has been reported to increase wtp53 protein expression. U266 MM cells express only mtp53, whereas ARH-77 and RPMI 8226 MM cells express both wtp53 and mtp53 protein (Fig 7A). OCI-My5 MM cells and cells from patient PCL1 weakly express both wtp53 and mtp53, and cells from patient PCL2 weakly express only mtp53. Normal BM MNCs weakly express wtp53 but not mtp53, and expression can be increased after 1 hour and 4 hours by XRT.

We next examined whether MDM2 was binding to wtp53 and/or mtp53 in RPMI 8226 MM cells. Both wtp53 and mtp53 were associated with MDM2 in these tumor cells (Fig 7B). In contrast, no wtp53- or mtp53-MDM2 protein complexes were detected in Saos-2 cells, which lack p53 protein, or in untreated or irradiated normal BM MNCs, which lack MDM2 expression.

Association of MDM2 with cell-cycle regulatory proteins in p53-positive and p53-negative MM-derived cell lines and PCL patient cells. Given the heterogeneity of p53 expression in MM cell lines and PCL patient cells demonstrated already, we next wanted to determine the impact of p53 expression on the association of MDM2 with other cell-cycle regulatory proteins. MDM2 protein was constitutively associated with CDK4, E2F-1, and p21 in all MM cell lines and PCL patient cells; however, constitutive binding of MDM2 to CDK4 was weak in all tumor cells, whereas MDM2–E2F-1 binding was strong in all tumor cells (Fig 8). Furthermore, cells that weakly express p53 (OCI-My5 and PCL1) demonstrate more MDM2–p21 complex formation compared with cells that strongly express p53 (ARH-77 and RPMI 8226), which demonstrate less MDM2–p21 binding. Immunoprecipitation and immunoblotting of these cell lysates using the same respective Abs served as positive controls.

DISCUSSION

In this study, we have shown that MDM2 protein is overexpressed in MM cell lines and PCL patient cells as com-
pression, we first confirmed the specificity of MDM2 antisense ODNs used in these studies, evidenced by abrogation of MDM2 protein expression in these tumor cells without associated alteration in the expression of p53, E2F-1, CDK4, or p21. In contrast to a previous report using U87-MG human glioblastoma cells, RPMI 8226 MM cells clearly demonstrate large amounts of MDM2 and both wt53 and mt53 protein constitutively, and MDM2 expression was inhibited by MDM2 antisense ODN at an ODN concentration of 1.0 to 2.0 μmol/L within 2 to 6 hours. We also confirmed that the half-life of MDM2 was approximately 25 minutes in RPMI 8226 cells, supporting the observed rapid and marked inhibition of MDM2 expression by MDM2 antisense ODN in these MM cells. These differences between results in U87-MG human glioblastoma cells and our results in RPMI 8226 MM cells suggest that the effects of MDM2 antisense ODN may be modulated by other factors, such as p53 status, in different cell lines.

We next showed that treatment with MDM2 antisense ODN, but not with MDM2 sense, nonsense, or scrambled ODN, was able to transiently decrease cell proliferation and viability at 2 to 4 hours, indicating specific biologic sequelae of the antisense ODN. Moreover, if antisense ODN was not replenished, recovery of proliferation and viability to baseline occurred within 8 to 16 hours, consistent with the short observed half-life of MDM2 in these MM cells. Our subsequent experiments replenished MDM2 antisense ODN every 4 hours and resulted in sustained enhanced antiproliferative effects, further demonstrating the specificity of biologic sequelae related to the anti-MDM2 antisense ODNs.

MDM2 antisense ODNs also induced G1 growth arrest and triggered apoptosis, assessed by PI staining and flow cytometric analysis. pRB was predominantly phosphorylated and not bound to E2F-1 in MM cells, allowing for G1 to S phase transition. However, binding of E2F-1 to pRB was induced by MDM2 antisense ODN, restoring G1 growth arrest. The observation that MDM2 sense, MDM2 nonsense, and MDM2 scrambled ODN cells continued to progress from G1 to S phase further supports the specific role of MDM2.
Fig 4. Effect of culture with MDM2 antisense ODN on proliferation in RPMI 8226 MM cells. RPMI 8226 MM cells were cultured in media with MDM2 antisense ODN (A15 and A20, A and E), MDM2 sense ODN (S15 and S20, B and F), MDM2 nonsense ODN (N15 and N20, C and G), or MDM2 scrambled ODN (X15 and X20, D and H) at concentrations of 0.1, 1.0, and 2.0 μmol/L for up to 24 hours, without replenishment of ODNs. In addition, RPMI 8226 MM cells were cultured with 1.0 μmol/L of each ODN, with replenishment of ODN every 4 hours. Proliferation was measured by ³H-TdR uptake at 0, 0.5, 1, 2, 4, 6, 8, 12, 16, 20, and 24 hours. Viable cell density at 0, 0.5, 1, 2, 4, 6, 8, 16, and 24 hours was also determined by trypan blue exclusion for RPMI 8226 MM cells cultured in 1.0 μmol/L of each ODN, without replenishment of ODN.
Fig 5. Effect of culture with MDM2 antisense ODN on cell-cycle distribution and apoptosis in RPMI 8226 MM cells. RPMI 8226 MM cells were cultured with MDM2 antisense ODN (A15), media alone, MDM2 sense ODN (S15), or MDM2 scrambled ODN (X15) at concentrations of 1.0 μmol/L for up to 24 hours, without replenishment of ODNs. Cell-cycle distribution (A to D) was defined by PI staining and flow cytometric analysis. The percentage of apoptotic cells (E to H) was determined by acridine orange–ethidium bromide staining.
**Fig 6.** Effect of culture with MDM2 antisense ODN on expression of MDM2, phosphorylation of pRB, and pRB–E2F-1 binding in RPMI 8226 MM cells. RPMI 8226 MM cells were cultured with 1.0 μmol/L MDM2 antisense ODN (A15) for up to 24 hours. Cell lysates prepared before and at 0.5, 1, 2, 4, 6, 8, 16, and 24 hours were immunoprecipitated with SMP-14 anti-MDM2 MoAb and blotted with the same Ab (A). Cell lysates were immunoprecipitated with Ab-1 C36 anti-pRB MoAb, followed by immunoblotting with Ab-6 AF11 anti-pRB MoAb (B) or C-20 anti–E2F-1 MoAb (C).

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**Fig 7.** Expression of wt-p53 and mt-p53, as well as p53-MDM2 binding, in MM-derived cell lines, PCL patient cells, Saos-2 osteosarcoma cells, and normal BM MNCs. Total cell lysates of MM cell lines (U266, ARH-77, RPMI 8226, and OCI-My5), PCL patient cells (PCL1 and PCL2), Saos-2 p53-deficient osteosarcoma cells, and normal BM MNCs (without or 1 or 4 hours post-XRT) were immunoprecipitated with Ab-5 anti-wt-p53 MoAb or Ab-3 anti-mt-p53 MoAb and immunoblotted with DO-1 hrp anti-p53 MoAb. Normal BM MNCs served as a positive control for wt-p53; U266 MM cells served as a positive control for mt-p53; and p53-deficient Saos-2 cells served as a negative control for both wt-p53 and mt-p53 (A). Total cell lysates of RPMI 8226, Saos-2 osteosarcoma cells, and normal BM MNCs (without or 1 or 4 hours post-XRT) were immunoprecipitated with Ab-5 anti-wt-p53 MoAb or Ab-3 anti-mt-p53 MoAb, followed by immunoblotting with SMP14 anti-MDM2 MoAb (B).
wtp53 and mtp53, respectively. Both wtp53 and mtp53 bind MDM2, confirmed in RPMI 8226 MM cells in this study, suggesting competition for a common p53 binding site. It has been shown that MDM2 binding to wtp53 inhibits wtp53 action on genes bearing specific p53 response elements (REs), and that the MDM2 gene has two tandem p53 REs in its intronic promoter. Binding of wtp53 can induce MDM2 gene transcription through activation of its p53 REs. This creates an autoregulatory feedback loop, whereby wtp53 enhances MDM2 expression and MDM2 then binds and inhibits wtp53. Hence, the presence of both wtp53 and mtp53 protein expression in the setting of MDM2 overexpression may interfere with this autoregulatory mechanism and is potentially tumorigenic: mtp53 binds to MDM2 and prevents wtp53–MDM2 complex formation, thereby allowing unopposed wtp53 effects. Our studies demonstrate coexpression of wtp53 and mtp53 in the setting of MDM2 overexpression in RPMI 8226 MM cells, highlighting the potential importance of the expression and function of these proteins in the pathogenesis of MM.

Finally, MDM2 is also known to associate with cell-cycle regulatory proteins other than p53, including pRB and the E2F-1/DP-1 complex. In our study, MDM2 expressed in MM cell lines and PCL patient cells binds E2F-1; in MM cells that lack p53, MDM2 also binds p21. p21 is activated by wtp53 and potentiates wtp53 tumor-suppressor function by strongly inhibiting cell-cycle regulatory proteins such as cyclin E and CDK2. The binding of MDM2 to p21, especially in MM cells lacking p53, may abrogate the inhibitory function of p21 and thereby facilitate MM cell growth. Our studies therefore suggest that inhibition of MDM2 may serve as a useful treatment strategy in MM.

Fig 8. Association of MDM2 with cell-cycle regulatory proteins in p53-positive and p53-negative MM-derived cell lines and PCL patient cells. Total cell lysates of MM cell lines (ARH-77, RPMI 8226, and OCI-My5) and PCL patient cells (PCL1 and PCL2) were immunoprecipitated with H-22 anti-CDK4 pAb, C-20 anti–E2F-1 MoAb, C-19 anti-p21 pAb, or SMP14 anti-MDM2 MoAb, followed by immunoblotting with SMP14 anti-MDM2 MoAb. These cell lysates were immunoprecipitated and immunoblotted using the same Abs as positive controls.

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