Inactivation of Tumor Suppressor p53 by Mot-2, a hsp70 Family Member

(Received for publication, May 22, 1998, and in revised form, July 28, 1998)

Renu Wadhwa‡§, Syuichi Takano¶, Martin Robert‡, Akiko Yoshidaí, Hitoshi Nomuraí, Roger R. Reddel, Youji Mitsuí, and Sunil C. Kaul

From the §Chugai Research Institute for Molecular Medicine, 153-2 Nagai, Niihara, Ibaraki 300-41, Japan, the ¶National Institute of Bioscience and Human Technology, AIST, 1-1 Higashi, Tsukuba, Ibaraki 305-8566, Japan, and the iChildren's Medical Research Institute, 214 Haukesbury Road, Westmead Sydney, New South Wales 2145, Australia

The mortalin genes, mot-1 and mot-2, are hsp70 family members that were originally cloned from normal and immortal murine cells, respectively. Their proteins differ by only two amino acid residues but exhibit different subcellular localizations, arise from two distinct genes, and have contrasting biological activities. We report here that the two proteins also differ in their interactions with the tumor suppressor protein p53. The pancytosolic mot-1 protein in normal cells did not show colocalization with p53; in contrast, nonpancytosolic mot-2 and p53 overlapped significantly in immortal cells. Transfection of mot-2 but not mot-1 resulted in the repression of p53-mediated transactivation in p53-responsive reporter assays. Inactivation of p53 by mot-2 was supported by the down-regulation of p53-responsive genes p21\textsuperscript{WAF-1} and mdm-2 in mot-2-transfected cells only. Furthermore, NIH 3T3 cells transfected with expression plasmid encoding green fluorescent protein-tagged mot-2 but not mot-1 showed an abrogation of nuclear translocation of wild-type p53. These results demonstrate a novel mechanism of p53 inactivation by mot-2 protein.

Evidence has been accumulating that inactivation of p53, a tumor suppressor and cellular transcription factor (1), is involved in cellular transformation and immortalization (2–5). Extensive analyses of p53 have defined at least four functional domains, including an amino terminus transactivation domain (amino acids 1–44), a sequence-specific DNA-binding domain (amino acids 100–300), a carboxyl terminus oligomerization domain, and a regulatory domain (amino acids 319–393; Ref. 6), and shown that the conformation of p53 and its interactions with other proteins have key roles in its various cellular activities (7, 8). Several cellular proteins, including some of the hsp70 family members, have been shown to interact with p53 (9–12). Although mutual or mdm-2-mediated inactivation of p53 is a common event involved in cellular transformation (1), p53 is inactivated in a considerable number of tumors and transformed cells by an unknown mechanism(s).

We initially cloned mortalins mot-1 and mot-2, which code for pancytosolically and perinuclearly distributed members of the hsp70 family of proteins, from normal and immortal murine cells, respectively (13, 14). The open reading frames of the two types of murine mortalins differ in two nucleotides, encode proteins differing in two amino acids, arise from distinct genes, and have contrasting biological activities (13–16). RNA in situ hybridization and immunohistochemical studies on mortalin in normal murine tissues showed a higher level of expression in nondividing cell populations than in dividing cells. However, tumor tissues were seen to have a high intensity of mortalin staining by an antibody that reacts with both the mot-1 and mot-2 proteins (17, 18). Mortalin was also identified as PBP-74, mtHSP70, and Grp75 and has been assigned roles in antigen processing, in vivo nephrotoxicity, and radioreistance in independent studies from other groups (19, 20).

In the present study, we demonstrate functional interactions of mot-2 protein with wild-type p53. Colocalization of wild-type p53 and mortalin protein was observed in transformed (nonpancytosolic mortalins) but not in normal (pancytosolic mortalins) cell types. The transcriptional activation function of p53 was impaired by transfections of mot-2 but not mot-1. Consistently, transfections of mot-2, but not those of mot-1, resulted in the down-regulation of p53-responsive genes p21\textsuperscript{WAF-1} and mdm-2. Furthermore, G\textsubscript{i}-associated nuclear translocation of p53 was abrogated by mot-2 but not by mot-1. These results demonstrated that mot-2 protein inactivates wild-type p53 function.

EXPERIMENTAL PROCEDURES

Cell Culture—Cells were cultured as described previously (Refs. 13, 14, and 21; American Type Culture Collection catalogue).

Immunofluorescence and Fluorescence Digital Imaging Microscopy—Cells were double-stained with monoclonal anti-p53 (PAb421; Calbiochem) and polyclonal anti-mortalin antibodies (13) and visualized by secondary staining with fluorescein isothiocyanate-conjugated sheep anti-mouse IgG and Texas Red-conjugated donkey anti-rabbit IgG (Amersham Corp.). Three-dimensional images with enhanced fluorescence were obtained using laser digital imaging microscopy with a ×40 Plan-NEOFLUAR objective on a Zeiss Axioshot microscope (Carl Zeiss, Germany) equipped with a CELScan system (Scanalytics, Billerica, MA; Ref. 22). The extent to which the two proteins were similarly distributed was assessed by combining the two images using computer graphics software. The individual mortalin and p53 images were seen as red and green fluorescence, respectively, and the colocalized proteins appeared yellow under this program.

Transfections—Transient and stable transfections were performed using LipofectAMINE\textsuperscript{TM} (Life Technologies, Inc.). Typically, 3 μg of plasmid DNA were used per 6-cm dish. All assays were performed after 48 h of transfections. Stable clones of NIH 3T3 cells with temperature-sensitive p53 expression were isolated by cotransfections of pMSVp53Val135 (a kind gift from Dr. Paul Jackson; Ref. 23) and a pSRα mammalian expression vector containing the hybrid SV40-human immunodeficiency virus promoter/enhancer and the neo\textsuperscript{G} gene and analyzed for p53 expression by Western blotting with anti-p53 antibody (PAb421).

This paper is available on line at http://www.jbc.org

* This work was supported in part by the Center of Excellence Grant, Japan (to S. C. K. and Y. M.) and the Carcinogenesis Fellowship of the New South Wales Cancer Council (to R. R. R.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed: Chugai Research Institute for Molecular Medicine, 153-2 Nagai, Niihara, Ibaraki 300-41, Japan. Tel.: 81-298-30-6211; Fax: 81-298-30-6270; E-mail: renu@tk.chugai-pharm.co.jp.
In Vivo Coimmunoprecipitation—Nonidet P-40 lysates (500 μg) from NIH 3T3 and NIH 3T3/p53.4 cells were precleared by incubating with 40 μl of protein A-agarose for 2 h. The supernatant was incubated with slow agitation overnight at 4 °C with 2–5 μg of control (isotype-matched IgG) or anti-p53 antibody (PAb421) that was cross-linked to protein A-agarose beads with dimethylpimelimidate as described previously (24). Immunocomplexes were pelleted by centrifugation, washed with Nonidet P-40 lysis buffer, separated by SDS-polyacrylamide gel electrophoresis, and transferred to nitrocellulose membrane by semidry transfer. The membrane was probed with an anti-mortalin antibody raised against a peptide (amino acids 469–487; Ref. 13).

Reporter Assays—Cells grown in 12-well plates were transfected with 0.5 μg of p53-responsive reporter plasmid, pWWP-luc or PG13-luc (kindly provided by Dr. Bert Vogelstein; Ref. 25), and 0.5 μg of empty vector (pSRα) or mot-1 (pSRα/mot-1) or mot-2 (pSRα/mot-2) expression plasmids. pMSVp53Val135 was cotransfected into p53−/− cells. Cotransfections of pRL-CMV were performed as an internal control to determine the efficiency of transfections. 48 h after transfection, luciferase assays (Dual-Luciferase Reporter Assay System; Promega) were performed. Luciferase values were calculated per microgram of the protein as determined by Bradford protein assay.

Western Blot Analysis—The protein sample (20–40 μg) separated on a SDS-polyacrylamide gel was electroblotted onto nitrocellulose membrane using a semidry transfer blotter. Immunoassays were performed with anti-mortalin, anti-p53 (PAb421), anti-mdm-2 (SMP14; Santa Cruz Biotechnology), anti-p21WAF-1 (C-19; Santa Cruz Biotechnology), anti-GFP1 (CLONTECH), and anti-actin (Boehringer Mannheim) antibodies.

RESULTS

Double Immunolocalization of p53 and Mortalin—Normal and immortal murine fibroblasts were double-stained for mortalin and p53. Whereas cytoplasmic staining of p53 was barely visible, nuclear p53 could be detected easily with an epifluorescence microscope (Fig. 1A). Next, the immunofluorescence was observed under a high-resolution three-dimensional digital laser microscope (Fig. 1B). Negative controls, including the staining of p53−/− fibroblasts and the staining of NIH 3T3 cells with secondary antibodies alone (data not shown), confirmed the specificity of the p53 immunostaining. Fluorescence digital imaging analysis revealed the colocalization of p53 and mortalin in the perinuclear region of NIH 3T3 cells that express mot-2; however, no colocalization was observed in normal mouse fibroblasts (CMEF) cells expressing pancytosolically distributed mot-1 protein (Fig. 1B). In contrast, normal human fibroblasts TIG-3 and MRC-5 (data not shown), which express wild-type p53 and pancytosolic mortal in, did not show any colocalization.

In Vivo Coimmunoprecipitation—Nonidet P-40 lysates (500 μg) from NIH 3T3 and NIH 3T3/p53.4 cells were precleared by incubating with 40 μl of protein A-agarose for 2 h. The supernatant was incubated with slow agitation overnight at 4 °C with 2–5 μg of control (isotype-matched IgG) or anti-p53 antibody (PAb421) that was cross-linked to protein A-agarose beads with dimethylpimelimidate as described previously (24). Immunocomplexes were pelleted by centrifugation, washed with Nonidet P-40 lysis buffer, separated by SDS-polyacrylamide gel electrophoresis, and transferred to nitrocellulose membrane by semidry transfer. The membrane was probed with an anti-mortalin antibody raised against a peptide (amino acids 469–487; Ref. 13).

Reporter Assays—Cells grown in 12-well plates were transfected with 0.5 μg of p53-responsive reporter plasmid, pWWP-luc or PG13-luc (kindly provided by Dr. Bert Vogelstein; Ref. 25), and 0.5 μg of empty vector (pSRα) or mot-1 (pSRα/mot-1) or mot-2 (pSRα/mot-2) expression plasmids. pMSVp53Val135 was cotransfected into p53−/− cells. Cotransfections of pRL-CMV were performed as an internal control to determine the efficiency of transfections. 48 h after transfection, luciferase assays (Dual-Luciferase Reporter Assay System; Promega) were performed. Luciferase values were calculated per microgram of the protein as determined by Bradford protein assay.

Western Blot Analysis—The protein sample (20–40 μg) separated on a SDS-polyacrylamide gel was electroblotted onto nitrocellulose membrane using a semidry transfer blotter. Immunoassays were performed with anti-mortalin, anti-p53 (PAb421), anti-mdm-2 (SMP14; Santa Cruz Biotechnology), anti-p21WAF-1 (C-19; Santa Cruz Biotechnology), anti-GFP1 (CLONTECH), and anti-actin (Boehringer Mannheim) antibodies.

1 The abbreviation used is: GFP, green fluorescent protein.
mine whether the colocalized proteins actually interact, p53 immunocomplexes from NIH 3T3 cells (which contain mot-2 protein) were Western blotted with anti-mortalin antibody. A faint band of mortalin was detected in these immunocomplexes. Control precipitations with isotype-matched IgG did not bring down mortalin protein. Quantitation of the input and immunoprecipitated mortalin signals revealed that approximately 0.7% of the total mortalin present in the lysate was coprecipitated with p53. This was consistent with the low amounts of p53 (undetectable by Western blotting) present in these cells. To substantiate these findings, we stably transfected NIH 3T3 cells with a temperature-sensitive p53 expression plasmid, pMSP53Val135. A clone (NIH 3T3/p53.4) isolated from these transfections expressed wild-type p53 at 32.5 °C and mutant p53 at 37 °C, as demonstrated by the accumulation of the latter on Western blotting with the p53-specific antibody PAb421 (Fig. 2A). Furthermore, p53-responsive reporter assays were performed on NIH 3T3/p53.4 cells maintained at 32.5 °C and 37 °C (Fig. 2B). PG13-luc containing a synthetic wild-type p53-responsive promoter that contains 13 repeats of wild-type p53 binding consensus sequence was transfected into these cells. A 3–4-fold higher activity was observed in NIH 3T3/p53.4 cells as compared with untransfected NIH 3T3 controls when the cells were maintained at 32.5 °C after the transfections. In contrast, the cells maintained at 37 °C showed p53 activity comparable to that of the control. This confirmed the wild-type and mutant conformations of exogenous p53 in the NIH 3T3/p53.4 clone at 32.5 °C and 37 °C, respectively. The clone was next used for p53 immunoprecipitation. Western blotting of the p53 immunocomplexes from NIH 3T3/p53.4 cells (grown at 32.5 °C) with antimortalin antibody revealed the presence of mortalin (mot-2) at a level that was much higher than that in NIH 3T3 cells (Fig. 2C). Quantitation of the input and immunoprecipitated mot-2 signals revealed that approximately 3.2% of the total mortalin present in the NIH 3T3/p53.4 lysate was immunoprecipitated. These data demonstrated a nearly 4.5-fold enrichment of the immunoprecipitated mot-2 protein in cells overexpressing p53 and therefore suggest that mot-2 and p53 bind in vivo.

Functional Inactivation of p53 by Mot-2—We next examined whether mot-2 can functionally inactivate p53 by carrying out p53-responsive reporter assays. NIH 3T3, COS7, and embryonic fibroblasts from a p53-null (p53−/−) mouse were used for p53-mediated reporter assays with PG13-luc plasmid. Both NIH 3T3 and COS7 cells showed the presence of transcriptionally active p53 (Fig. 3A). Cotransfections of the expression plasmid (pSRα/mot-2) encoding mot-2 resulted in the decline of p53-responsive reporter activity. In contrast, mot-1 cotransfections did not show any effect. Next, the p53−/− cells were transfected with a temperature-sensitive p53 expression plasmid, pMSP53Val135, together with a wild-type p53-responsive reporter plasmid, either pWWP-luc containing the native p53-responsive promoter or PG13-luc. As expected, the cells maintained at 37 °C showed no transactivation (data not shown) of the reporter plasmids. Cotransfections of the p53 and mot-2 expression plasmids resulted in a significant decline of pWWP-luc (Fig. 3B) and PG13-luc (data not shown) activities at 32.5 °C. Furthermore, a dose-dependent effect of mot-2 plasmid concentration on reporter repression was observed. Cotransfections of the mot-1 expression plasmid, pSRα/mot-1, did not affect p53-mediated transactivation (Fig. 3B).

Inactivation of p53 by mot-2 but not mot-1 was supported by the analysis of p53-responsive genes in cells that were transiently transfected with expression plasmids encoding mot-1 or -mot-2 proteins tagged with GFP. The expression of exogenous mortalins was first analyzed by Western blotting with anti-GFP antibody (data not shown) and anti-mortalin antibody (Fig. 3C). The cell lysates were subsequently analyzed for p53 and p53-responsive genes mdm-2 and p21WAF-1 by Western blotting with specific antibodies. Cells transfected with mot-2 exhibited a lower level of steady-state expression of p53, mdm-2, and p21WAF-1 as compared with those transfected with the empty vector or mot-1 (Fig. 3C). These data were consistent with the inactivation of p53 by mot-2 but not mot-1 seen in the abovementioned reporter assays. The data also suggest that mot-2 acts, at least in part, by reducing the p53 steady-state levels.

Ablation of Nuclear Translocation of p53 by Mot-2—Wild-type p53 has been reported to translocate to the nucleus at the G1 stage of the cell cycle. It exhibits bright immunofluorescence in the nucleus as compared with the cytoplasm, where it is barely detected by epifluorescence microscopy (Fig. 1A). Immunolocalization of mot-2 and p53 in transformed cells

![Fig. 2. Coimmunoprecipitation of p53 and mot-2. A, exogenous expression of temperature-sensitive p53 in NIH 3T3 cells. A Western blot of NIH 3T3 and NIH 3T3/p53.4 cells (grown at 32.5 °C and 37 °C) with p53-specific antibody PAb421 and anti-actin antibody is shown. NIH 3T3/p53.4 cells grown at 37 °C showed the expected accumulation of mutant p53 at 37 °C. B, p53-responsive reporter assays in NIH 3T3 cells and its derivative cell line, NIH 3T3/p53.4. Cells were transfected with PG13-luc and pRL-CMV (containing Renilla luciferase reporter, an internal control for transfection efficiency) and maintained at 32.5 °C or 37 °C. A 3–4-fold higher reporter activity was detected when cells were maintained at 32.5 °C. Bars and error bars represent the mean ± S.D. (n = 3). Similar results were obtained in four experiments. C, coimmunoprecipitation of mot-2 with p53 in NIH 3T3 and NIH 3T3/p53.4 cells extracts grown at 32.5 °C. p53 immunocomplexes from the indicated cells were Western blotted with anti-mortalin antibody. Quantitation of the input (10% of the amount used for immunoprecipitation is seen) and the immunoprecipitated mortalins were performed by using Micro Computer Imaging Device MCID-M2 (FUJIX). 0.7 and 3.2% of total mot-2 were seen to coimmunoprecipitate with p53 in NIH 3T3 and NIH 3T3/p53.4 cells, respectively.

![Image](https://example.com/fig2.png)
prompted us to investigate whether the mot-2-mediated inactivation of p53 was accompanied by its retention in cytoplasm. Expression plasmids encoding fusion proteins containing GFP and either mot-1 or mot-2 were transiently transfected into NIH 3T3 cells grown on coverslips. Expression of the fusion proteins was confirmed by Western analysis with anti-GFP and anti-mortalin antibodies. The transfected cells were serum-starved for 48 h and stained for p53. Cytoplasmic p53 (detected as a very faint staining with Texas Red-conjugated secondary antibody) translocated to the nucleus (brighter staining) upon serum starvation in untransfected background cells. Of 155 cells that exhibited green fluorescence for mot-2-GFP, 139 (approximately 90%) were seen to have no nuclear staining of p53. On the contrary, of 89 mot-1-GFP-transfected cells, 78 (87.6%) were seen to have nuclear p53 similar to that of the untransfected cells in the same cultures. Thus, the transfected cells that expressed mot-2 (green fluorescence) were significantly devoid of nuclear p53 (red fluorescence; Fig. 4), demonstrating the abrogation of its translocation. Parallel experiments with mot-1-GFP did not reveal an equivalent effect in three independent experiments in which ~100–150 transfected cells were examined. The data were consistent with the abovementioned analysis on p53-responsive exogenous reporter assays and endogenous gene expression in mot-2- and mot-1-transfected cells and suggested that mot-2 interacts with wild-type p53 and causes its inactivation both by a reduction in its steady-state level and by nuclear exclusion.

DISCUSSION

Double immunolocalization studies with laser digital microscopy showed the colocalization of p53 and mot-2 in NIH 3T3 cells that have wild-type p53 (26–29). The faint staining of p53 observed by epifluorescence microscopy (Fig. 1A) and the undetectable amounts of p53 in these cells on Western blots (Fig. 2A) were consistent with a low-level expression of the wild-type protein. A perinuclear staining pattern for p53 has also been reported in immortalized but untransformed Balb/3T3 cells (30). The colocalization of mot-2 and p53 suggested that these proteins might interact, and this was confirmed by in vivo coimmunoprecipitations of the two proteins from NIH 3T3 and its transfected derivative that overexpressed wild-type p53.

COS7 cells showed detectable amounts of p53 by Western blotting, and p53 immunocomplexes from these cells were seen to have mortalin by Western blot analysis (data not shown). This supported the in vivo interactions of the two proteins. p53-responsive reporter assays in COS7 cells revealed that p53 is transcriptionally active in these cells. Several other studies have detected the presence of p53 that is unbound to SV40 T antigen in SV40-transformed cells (31–34). Furthermore, similar to NIH 3T3 cells, transfections of mot-2 but not mot-1 were seen to repress p53 activity in COS7 cells. Reporter assays performed on p53−/− mouse embryonic fibroblasts revealed repression of exogenous p53-mediated reporter activity by cotransfections of mot-2. In parallel experiments with mot-1, no

2 Unpublished data.
effect on p53 reporter activity was observed. Analysis on p53-responsive genes p21WAF-1 and mdm-2 exhibited down-regulation in mot-2-transfected cells, but not in mot-1-transfected cells. These data demonstrated the inactivation of wild-type p53 by mot-2. Consistent with the double immunolocalization data and the p53-responsive reporter assays, mot-1 did not show any effect on p53-responsive endogenous gene expression.

Immunolocalization studies on serum-deprived NIH 3T3 cells transfected with GFP-tagged mot-1 and mot-2 proteins showed abrogation of its nuclear translocation by mot-2. The differential effects of mot-1 and mot-2 in this respect were consistent with the results obtained in the above-described p53 transactivation and p53-responsive gene analysis. Such functional impairment of wild-type p53 may abrogate some or all of its normal tumor suppressor activity and therefore may contribute, at least in part, to the malignant transformation of mot-2-overexpressing NIH 3T3 cells (16). An elevated level of mortalin has been observed in rat and human brain tumors (17, 18), suggesting that up-regulation of mot-2 may be an event that contributes to tumor growth and/or progression. In addition, a reduced amount of steady-state p53 was detected in NIH 3T3 (data not shown) and COS7 (Fig. 3C) cells that were transfected with the mot-2 expression plasmid. The data suggested that mot-2 may cause either p53 degradation or transcriptional repression. Direct interactions of p53 and mot-2 (Fig. 2C), along with the predicted chaperonin function of the latter, suggested that it is more likely to be the result of mot-2-mediated p53 degradation.

Functional inactivation of wild-type p53 by abnormal sequestration in the cytoplasm has been reported in a subset of neuroblastomas (35). The mechanism of this sequestration has not been elucidated. Our immunofluorescence results (as described above) demonstrated that mortalin was colocalized with p53 in the cytoplasm of neuroblastoma (SY-5Y and YKG-1), glioblastoma (A172), teratocarcinoma (NT-2), cervical carcinoma (HeLa), bladder carcinoma (A2182), and osteosarcoma (U2OS) cells, all of which have wild-type p53, and suggested that mot-2 may be involved in the inactivation of wild-type p53 function in these cells. Ostermeyer et al. (36) have reported that the carboxyl terminus of p53 is involved in cytoplasmic aggregates in neuroblastomas. Interestingly, in in vitro pull down assays of mot-2 and in vitro-translated p53, a fragment of p53 protein that lacks the carboxyl terminus was not coimmunoprecipitated with mortalin in in vitro binding assays,\(^3\) suggesting that the carboxyl terminus of p53 may be involved in its interactions with mortalin. These observations are particularly interesting in the context of the presence of three nuclear localization signals in the carboxyl terminus of p53 (37) and may provide an explanation, at least in part, to the mot-2-mediated abrogation of the nuclear localization of p53.

In summary, mot-2 was shown to colocalize with wild-type p53 in vivo and to inhibit p53-mediated exogenous and endogenous transactivation. The mot-2-mediated reduction in the steady-state level and the abrogation of nuclear translocation of p53 suggested a novel mechanism of p53 inactivation that may contribute to tumorigenesis.

Acknowledgments—We thank Bert Vogelstein and Paul Jackson for the kind gifts of pWWP-luc and PG13-luc and mMSVp53Val135 plasmids, respectively.

REFERENCES

1. Levine, A. J. (1997) Cell 88, 323–331
2. Baker, S. J., Markowitz, S., Fearon, E. R., Willson, J. K. U., and Vogelstein, B. (1990) Science 249, 912–915
3. Harvey, D. M., and Levine, A. J. (1991) Genes Dev. 5, 2373–2385
4. Metz, T., Harris, A. W., and Adams, J. M. (1995) Cell 82, 29–36
5. Vermonique, G., and Wynford-Thomas, D. (1998) Mol. Cell. Biol. 18, 1611–1621
6. Harris, C. C. (1998) J. Natl. Cancer Inst. 88, 1442–1455
7. Adler, V., Pincus, M. R., Minamoto, T., Fuchs, S. S., Dietz, U., Bluth, M. J., Brantl-Rauf, P. W., Friedman, F. K., Robinson, R. C., Chen, J. M., Wang, X. W., Harris, C. C., and Romai, Z. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 1686–1691
8. Donehower, L. A., and Bradly, A. (1993) Biochem. Biophys. Acta 1155, 181–205
9. Clarke, C. F., Cheng, K., Frey, A. B., Stein, R., Hinds, P. W., and Levine, A. J. (1989) Mol. Cell. Biol. 9, 1206–1215
10. Hall, P. A., Meek, D., and Lane, D. (1996) J. Pathol. 180, 1–5
11. Merrick, B. A., He, C., Witcher, L. L., Patterson, R. M., Reid, J. J., Penge-Pawlowski, P. M., and Selkirk, J. K. (1996) Biochem. Biophys. Acta 1297, 57–68
12. Sugito, K., Yamane, M., Hattori, H., Hayashi, Y., Toshnoi, I., Ueda, M., Tsuchida, N., and Ohtsuka, K. (1995) FEBS Lett. 356, 161–164
13. Wadhwa, R., Kaul, S. C., Ikawa, Y., and Sugimoto, Y. (1993) J. Biol. Chem. 268, 6615–6621
14. Wadhwa, R., Kaul, S. C., Sugimoto, Y., and Mitsu, Y. (1993) J. Biol. Chem. 268, 22239–22242
15. Wadhwa, R., Kaul, S. C., Sugimoto, Y., and Mitsu, Y. (1993) J. Biol. Chem. 268, 22239–22242
16. Wadhwa, R., Akita, S., Sugihara, T., Reddel, R. R., Mitsu, Y., and Kaul, S. C. (1996) Exp. Cell Res. 226, 381–386
17. Kaul, S. C., Duncan, E. L., Englezou, A., Takano, S., Reddel, R. R., Mitsu, Y., and Wadhwa, R. (1998) Oncogene 16, 907–911
18. Kaul, S. C., Matsu, M., Takano, S., Sugihara, T., Mitsu, Y., and Wadhwa, R. (1997) Exp. Cell Res. 232, 56–63
19. Wadhwa, R., Yashii, Y., Nose, T., Kaul, S. C., and Mitsu, Y. (1997) Exp. Cell Res. 237, 38–45
20. Kaul, S. C., Mitsu, Y., and Wadhwa, R. (1998) Indian J. Exp. Biol. 36, 345–352

\(^3\) Unpublished observations.
Inactivation of p53 by mot-2 Protein

29591

20. Sadekova, S., Lehnert, S., and Chow, T. V. (1997) Int. J. Radiat. Biol. 72, 653–660
21. Wadhwa, R., Pereira-Smith, O. M., Reddel, R. R., Sugimoto, Y., Mitsui, Y., and Kaul, S. C. (1995) Exp. Cell Res. 216, 101–106
22. Fay, F. S., Carrington, W., and Fogarty, K. E. (1989) J. Microsc. 153, 133–149
23. Reed, M., Wang, Y., Mayr, G., Anderson, M. E., Schwedes, J. F., and Tegtmeyer, P. (1993) Gene Expr. 4, 95–107
24. Harlow, E., and Lane, D. (1988) Antibodies: A Laboratory Manual, pp. 521–523, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
25. El-Deiry, W. S., Tokino, T., Velculescu, V. E., Levy, D. B., Parsons, R., Trent, J. M., Lin, D., Mercer, D. W. E., Kinzler, K. W., and Vogelstein, B. (1993) Cell 75, 817–825
26. Kaul, S. C., Wadhwa, R., Sugihara, T., Obuchi, K., Komatsu, Y., and Mitsui, Y. (1994) Biochim. Biophys. Acta 1201, 389–396
27. Ozburn, M. A., Jerry, D. J., Kitrell, F. S., Medina, D. M., and Butel, J. S. (1993) Cancer Res. 53, 1646–1652
28. Rittling, S. R., and Denhardt, D. T. (1992) Oncogene 7, 935–942
29. Tokumitsu, M., Kadohama, T., and Ogawa, K. (1994) Mol. Carcinog. 10, 52–57
30. Milner, J., and Cook, A. (1986) Virology 150, 265–269
31. Deppert, W., and Huang, M. (1986) Mol. Cell. Biol. 7, 4453–4463
32. Kierstead, T. D., and Tevethia, M. J. (1993) J. Virol. 67, 1817–1829
33. Michael-Michalovitz, D., Yehiely, F., Gottlieb, E., and Oren, M. (1991) J. Virol. 63, 4160–4168
34. O'Neill, F. J., Hu, Y., Chen, T., and Carney, H. (1997) Oncogene 14, 955–965
35. Moll, U. M., Ostermeyer, A. G., Haladay, R., Winkfield, B., Frazier, M., and Zambetti, G. (1996) Mol. Cell. Biol. 16, 1126–1137
36. Ostermeyer, A. G., Runko, E., Winkfield, B., Ahn, B., and Moll, U. M. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 15190–15194
37. Kubbutat, M. H. G., and Vousden, K. H. (1998) Mol. Med. Today 4, 250–256