JOURNAL OF VIROLOGY, June 2006, p. 5644–5650 Vol. 80, No. 11
0022-538X/06/$08.00+0 doi:10.1128/JVI.02400-05

Measles Virus V Protein Inhibits p53 Family Member p73

Cristian D. Cruz,[1]† Heidi Palosaari,[1]† Jean-Patrick Parisien,[1]† Patricia Devaux,[2] Roberto Cattaneo,[2] Toru Ouchi,[3] and Curt M. Horvath[1]*

Department of Medicine and Department of Biochemistry, Molecular Biology, and Cell Biology, Northwestern University, Evanston, Illinois, and Department of Medicine, Evanston Northwestern Healthcare, Evanston, Illinois 60208[1]; Molecular Medicine Program, Mayo Clinic College of Medicine, Rochester, Minnesota 55905[2]; and Department of Oncological Sciences, Mount Sinai School of Medicine, New York, New York 10029[3]

Received 15 November 2005/Accepted 11 March 2006

Paramyxovirus V proteins function as host interference factors that inactivate antiviral responses, including interferon. Characterization of cellular proteins that copurify with ectopically expressed measles virus V protein has revealed interactions with DNA binding domains of p53 family proteins, p53 and p73. Specific transcriptional assays reveal that expression of measles virus V cDNA inhibits p73, but not p53. Expression of measles virus V cDNA can delay cell death induced by genotoxic stress and also can decrease the abundance of the proapoptotic factor PUMA, a p73 target. Recombinant measles virus with an engineered deficiency in V protein is capable of inducing more severe cytopathic effects than the wild type, implicating measles virus V protein as an inhibitor of cell death. These findings also suggest that p73-PUMA signaling may be a previously unrecognized arm of cellular innate antiviral immunity.

The tumor suppressor p53 is one member of a protein family that includes the homologues p73 and p63 (reviewed in reference 19). These proteins share domain architecture and exhibit substantial amino acid sequence homology with p53, most prominently in the DNA binding domain. The p73 and p63 proteins play essential but distinct roles in cellular homeostasis and mammalian development. p73 is thought to be a primary inducer of the mitochondrial apoptosis pathway, functioning as a transcriptional regulator of genes such as PUMA (*p*53 *u*pregulated *m*ediator of *a*poptosis), and can also activate transcription of the Bax gene promoter (18). The interrelationships between the p53 family members are complex, as combinatorial deficiencies for these p53 family members have revealed unexpected cooperativity in their regulation of apoptotic responses and tumor development (7, 8).

In cases of viral oncogenesis, p53 has been demonstrated to interact with proteins encoded by tumor-associated viruses, many times providing a host evasion mechanism for bypassing cell cycle arrest and/or apoptosis (22). Destruction of this important cancer elimination checkpoint abrogates tumor suppressor activity in the infected cells and contributes to the oncogenic properties of tumor viruses. Connections between the p53 family and innate antiviral immunity have been recently established. Evidence indicates p53 pathways may be components of the antiviral responses mediated by interferon (IFN) (17, 35). Transcription of the p53 gene is induced by IFN, accompanied by an increase in p53 protein levels that prime cells toward enhanced p53 responses. The p53 protein can be activated in response to further virus infection, inducing a cellular apoptotic response that can restrict virus replication. Thus, there exists a relationship between death-inducing stress responses and innate antiviral immunity that transcends the tumor suppression properties of p53. This connection is also supported at the level of gene expression, as several gene targets of the IFN system are also subject to regulation by the p53 family (e.g., see references 2, 13, 24, and 25). Furthermore, influenza virus induces p53 activity, including p53-dependent apoptosis (37, 38), and cells deficient in p53 are defective in IFN responses (12, 38). In some cases, the viral protein that targets p53 is also linked to evasion of host innate immunity. For example, human papillomavirus E6 protein can enhance the ubiquitylation and degradation of p53 (32) and also can inhibit the induction of IFN through interaction with IFN regulatory factor 3 (31). Altogether, these findings support a role for the p53 family pathways in cellular regulation of virus infections.

Measles virus, a member of the *Morbillivirus* genus in the *Paramyxoviridae* family, is an enveloped negative-strand RNA virus that causes an acute illness, typically during childhood. Measles infections can promote a virus-induced immune suppression that creates susceptibility to opportunistic infections, resulting in over 1 million deaths annually worldwide (21). Measles virus disengages the host innate and adaptive antiviral immune systems in several ways. Like many other paramyxoviruses, measles virus encodes a protein, termed "V," that functions as an inhibitor of host innate antiviral responses. Paramyxovirus V proteins are key mediators of host evasion and have been demonstrated to disengage IFN signal transduction, to prevent IFN biosynthesis, to regulate host range restriction, and to interfere with virus-induced cell death (4, 5, 9, 10, 27, 28, 30, 45, 46). For example, recombinant simian virus 5 (SV5) viruses with specific defects in V protein expression are potent inducers of cell death, while the wild-type virus is not (10, 34, 43, 44). The measles virus V protein binds to a number of cellular proteins, including the IFN signaling proteins, STAT1 and STAT2 (26), allowing for efficient evasion of the host's IFN-induced antiviral immune response. Here, we demonstrate that the p53 family is also a target for measles

* Corresponding author. Mailing address: Pancoe-ENH Research Pavilion, Northwestern University, 2200 Campus Dr., Evanston, IL 60208. Phone: (847) 491-5530. Fax: (847) 491-4400. E-mail: Horvath@northwestern.edu.

† C.D.C., H.P., and J.-P.P. contributed equally to this study.

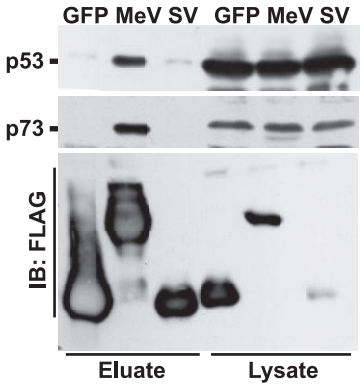

FIG. 1. Measles virus V protein binds to p53 and p73. Immunoaffinity purification of FLAG-tagged GFP, measles virus V (MeV), or SV5 V (SV) expressed in human 293T cells with FLAG M2 affinity resin was carried out as described previously (39). Whole-cell lysates and FLAG tripeptide eluates were subject to immunoblotting (IB) with antiserum for p53, p73, or FLAG epitope as indicated.

virus V host interference that enables evasion of programmed cell death.

Paramyxovirus V proteins exert anticellular actions through protein-protein interactions (1, 11, 14, 15). Affinity chromatography was employed previously to identify cellular proteins that interact with measles virus V (26, 36), and two prominent species were identified as p53 and p73 by specific immunoblotting (Fig. 1). A control FLAG-tagged green fluorescent protein (GFP) did not bind to p53 or p73, and the V protein from parainfluenza virus 5 (SV5) precipitated only a trace of p53 and no p73. However, the FLAG-measles virus V eluate contained both p53 and p73α (Fig. 1). These results indicate that both p53 and p73α are able to associate specifically with the measles virus V protein.

Proteins in the p53 family share a fundamental domain architecture consisting of an N-terminal transcriptional activation domain, a proline-rich region, a DNA binding domain, and a C-terminal region (see Fig. 2A). To more precisely define the site of interaction with measles virus V, expression vectors encoding epitope-tagged p53 and p73 fragments were expressed in cells and examined for measles virus V interaction. Full-length p53 (fragment i), a central fragment encompassing amino acids 43 to 300 (fragment ii), and the isolated DNA binding domain fragment (residues 100 to 300; fragment iii) were all able to coprecipitate with measles virus V, but the C-terminal domain (residues 300 to 393; fragment iv) was not (Fig. 2A). These experiments demonstrate that the p53 DNA binding domain mediates the measles virus V association.

Similar coprecipitation experiments were performed with epitope-tagged full-length p73 (fragment v) and a p73 DNA binding domain fragment (fragment vi) (Fig. 2B). Both the full-length p73 and the isolated p73 DNA binding domain (residues 116 to 320) were able to coprecipitate with measles virus V protein. Together, the findings indicate that the DNA binding domains of both p73 and p53 contain sites of interaction with the measles virus V protein.

Although p53 and p73 mediate distinct functions in mammals, they overlap in their abilities to induce programmed cell death. To test the ability of measles virus V to interfere with

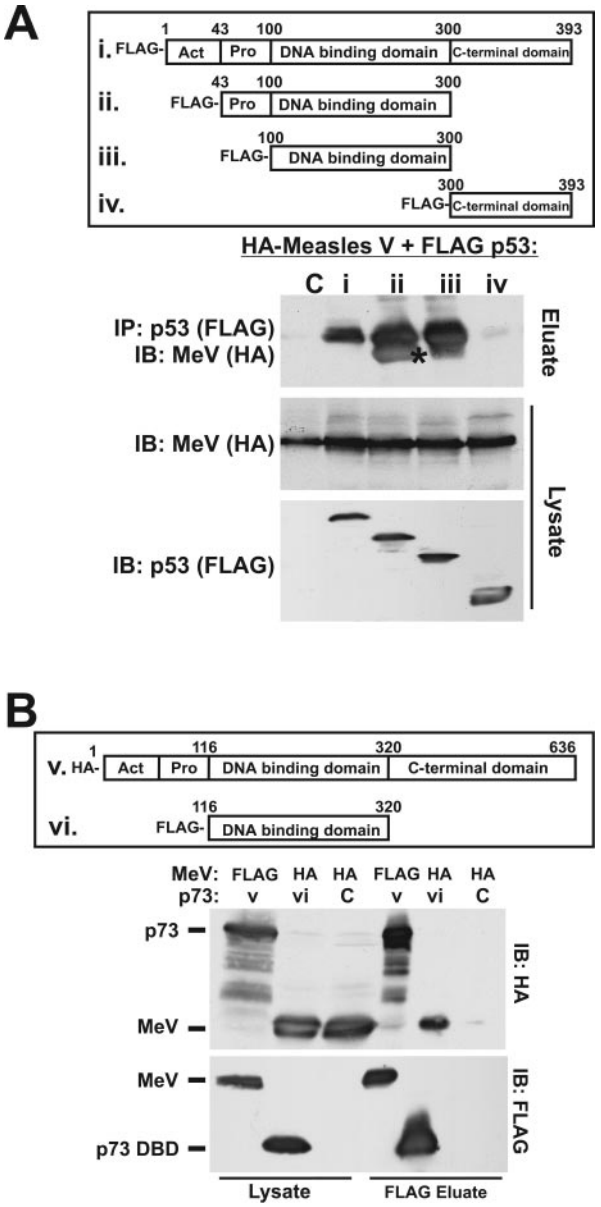

FIG. 2. Measles virus V targets p53 and p73 DNA binding domains. (A) FLAG-tagged p53 (i) and the indicated fragments (ii, iii, and iv) illustrated in the box were expressed in 293T cells along with hemagglutinin (HA)-tagged measles virus V (MeV), and complexes were captured by immunoprecipitation (IP) with FLAG M2 affinity resin. Whole-cell lysates and FLAG tripeptide eluates were subjected to immunoblotting (IB) for HA tag to detect measles virus V or FLAG tag to detect p53. An asterisk indicates a nonspecific cross-reactivity smear due to closely migrating fragment ii. (B) HA- or FLAG-tagged p73 (v) or p73 DNA binding domain fragment (vi) illustrated in the box was expressed with FLAG- or HA-tagged measles virus V. The left side shows proteins before immunoaffinity precipitation. The right panel shows proteins collected with FLAG M2 affinity resin, eluted with FLAG tripeptide, and subjected to immunoblotting for HA (top panel) or FLAG (bottom panel) as indicated.

apoptosis, parental 2fTGH fibrosarcoma cells and measles virus V-expressing derivatives were generated using replication-defective lentivirus vectors. These cell lines are defective in IFN signaling, consistent with prior conclusions regarding in-

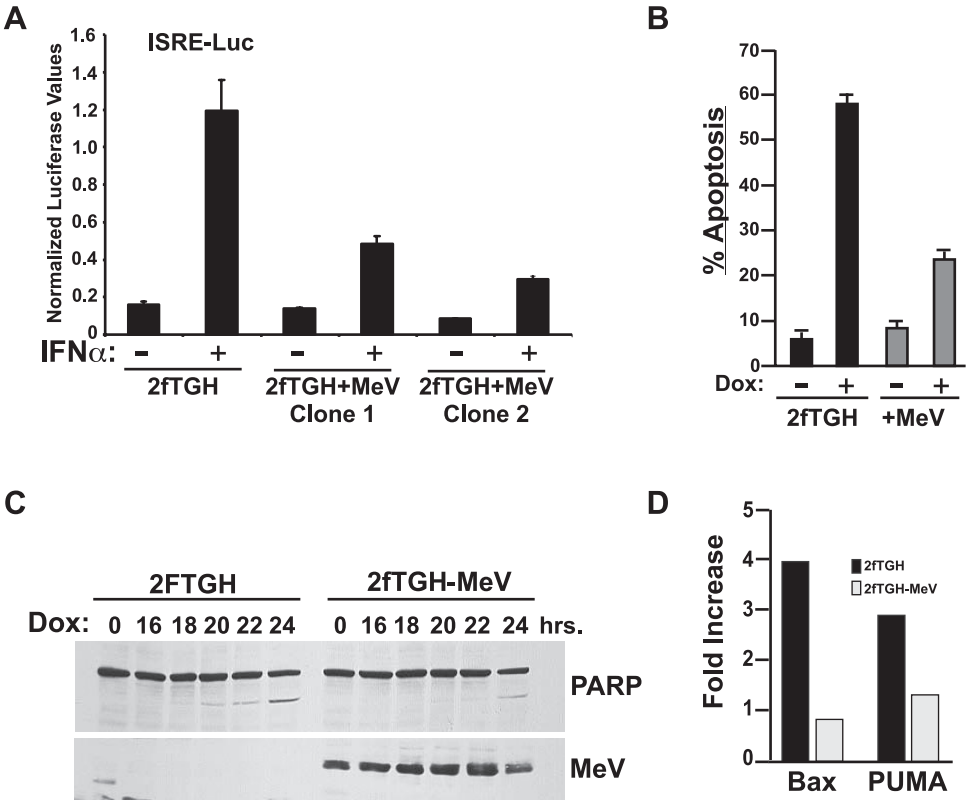

FIG. 3. Measles virus V protein expression suppresses cell death and alters accumulation of proapoptotic mRNAs. (A) Functional expression of measles virus V in stable cell lines. Human fibrosarcoma cell line 2fTGH and two measles virus V-expressing derivatives made using recombinant lentivirus vectors as described previously (40) using the pLenti system were subjected to IFN-α-responsive luciferase assays to verify the ability of measles virus V to suppress IFN signaling. Cells were treated with 1,000 U/ml IFN-α for 6 h beginning 24 h after reporter transfection and then lysed. (B) Analysis of DNA fragmentation. Human fibrosarcoma cell line 2fTGH and the measles virus V-expressing derivative were left untreated (−) or were treated with Dox (+; 1 μg/ml) for 24 h and then stained with propidium iodide. The fraction of cells exhibiting sub-G_0 DNA content is indicated as percent apoptosis. Average values with standard deviations are graphed ($n = 3$). (C) Analysis of PARP cleavage. 2fTGH cells or measles virus V-expressing cells were treated with doxorubicin (0.5 μg/ml) for the times (h) indicated, and whole-cell extracts were subjected to immunoblotting with antiserum for PARP or FLAG (to detect measles virus V protein). (D) Analysis of mRNA accumulation in response to Dox treatment. Focused gene expression DNA arrays for human p53-related genes were obtained from SuperArray Biosciences and probed and analyzed according to the manufacturer's recommendations using total RNA from 2fTGH and measles virus V-expressing derivative with and without Dox treatment. Cells were treated for 16 h with 1 μg/ml doxorubicin or left untreated, and data are the average increase (fold) by Dox treatment of three independent experiments.

hibition of JAK-STAT signaling (Fig. 3A) in the presence of measles virus V (26). These cell lines were left untreated or were treated with doxorubicin (Dox) to induce the DNA damage response (47). The cellular DNA content was analyzed by propidium iodide staining and flow cytometry (Fig. 3B). DNA fragmentation was induced by Dox, but the measles virus V-expressing cells were protected from apoptosis compared to wild-type cells, as indicated by the reduction in sub-G_0 DNA. To verify this result at the molecular level, immunoblotting was used to detect cleavage of the poly(ADP-ribose) polymerase (PARP), another hallmark of apoptosis (Fig. 3C). In 2fTGH cells, the 89-kDa PARP cleavage fragment was initially detected after 20 h of treatment with 0.5 μg/ml Dox and accumulated thereafter. In contrast, expression of measles virus V delayed initial detection of cleaved PARP to 24 h, indicating delayed apoptosis induction.

To dissect the measles virus V-dependent apoptosis inhibition, expression of p53-related genes was analyzed using DNA arrays (Fig. 3D). Comparing the relative abundance of mRNAs induced by genotoxic stress in parental 2fTGH cells versus measles virus V-expressing 2fTGH cells revealed few genes that were differentially induced. However, at least two genes encoding apoptosis regulators, BAX and PUMA, were underrepresented in the measles virus V-expressing cells, consistent with the observed inhibition of cell death. These data suggest that measles virus V may inhibit apoptosis by preventing induction of a subset of p53 or p73 target genes.

To test the ability of measles virus V to specifically influence p53 transcriptional activity, reporter gene assays were carried out in human 293T cells. Expression of p53 can potently activate a luciferase reporter gene carrying a multimeric canonical p53/p73 response element. Expression of measles virus V did not diminish the transcriptional response due to p53 expression (Fig. 4A). In fact, enhanced reporter gene activity was observed when measles virus V was coexpressed with p53 in this assay. To validate this result, endogenous p53 activity was induced with a specific p53 agonist, Nutlin 3 (42), which disrupts the p53 binding site of Mdm2 (Fig. 4B). By activating p53

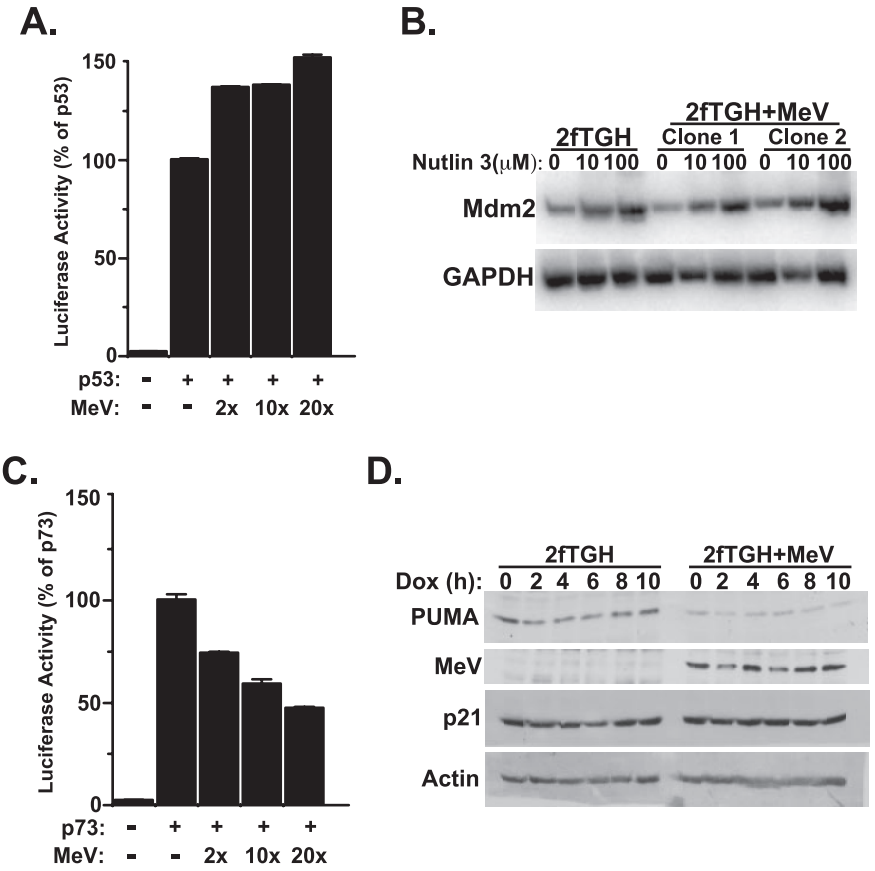

FIG. 4. Measles virus V selectively inhibits p73-mediated transcription and PUMA expression. (A) Effects of measles virus V (MeV) on p53-mediated gene regulation. Cells were cotransfected with p53 luciferase reporter plasmid, p53 expression vector, and measles virus V expression vector in the ratios indicated and assayed 36 h later. The data plotted are normalized to a cotransfected *Renilla* luciferase control, expressed as percent p53 activity alone ($n = 3$). (B) Activity of endogenous p53 on Mdm2 mRNA accumulation. 2fTGH and two measles virus V-expressing derivative clones were treated with the indicated concentrations of Nutlin 3 for 12 h, and then total RNA was processed for reverse transcription-PCR (RT-PCR) with specific primers for Mdm2 and GAPDH. (C) Measles virus V suppresses p73-mediated gene regulation. Cells were cotransfected with p73 reporter plasmid, p73 expression vector, and measles virus V expression vector in the ratios indicated and assayed 36 h later. Data plotted are normalized to a cotransfected *Renilla* luciferase control, expressed as percent p73 activity alone ($n = 3$). (D) Analysis of measles virus V effects on PUMA protein expression. 2fTGH and measles virus V-expressing derivative were treated with Dox (0.5 μg/ml) for the times indicated, and lysates were subjected to immunoblotting with specific antisera for PUMA, p21, α-actin, and FLAG (to detect measles virus V).

accumulation, Nutlin 3 treatment induced accumulation of Mdm2 mRNA (a p53 gene target) regardless of measles virus V expression. This result confirms that p53 activity is not inhibited by measles virus V protein, excluding p53 as an endogenous target for measles virus V protein interference.

In contrast to the observed inability of measles virus V to block p53-dependent transcription, measles virus V expression significantly inhibited luciferase activity due to p73 expression (Fig. 4C). Expression of p73 potently activated the reporter gene, but V protein expression resulted in dose-dependent inhibition, reducing luciferase activity by 50 to 60%. These data suggest that unlike p53, p73 is an authentic target for measles virus V host interference.

Expression of the PUMA protein is regulated by p73 during apoptosis induction (18). To determine the effect of measles virus V on endogenous p73 responses, the abundance of PUMA protein was tested by immunoblotting (Fig. 4D). PUMA was readily detected in parental 2fTGH cells, and its abundance remained relatively steady during 10 h of doxorubicin treatment. In contrast, detection of PUMA was diminished in cells harboring measles virus V protein. No changes were observed in the abundance of mRNAs encoding p21WAF1 or α-actin. Together, these data support the concept that p73 is a target of measles virus V protein interference with apoptosis.

To assess whether measles virus V protein acts as an inhibitor of cell death in a natural context, recombinant-derived wild-type and V-deficient measles viruses (named MVvac and MVvacVko, respectively) (3) were grown and their titers were determined in Vero cells for use in assays for virus-induced cytopathic effects (Fig. 5). It has been previously documented that apoptosis has a significant contribution to measles virus-induced cytopathicity (6). 2fTGH cells were inoculated with equal amounts of serially diluted viruses and assayed 3 days later by fixation and staining with methylene blue. While both viruses induced dose-dependent cytopathic effects, the V-deficient measles virus was more cytopathic than the wild type. To reduce any potential complications due to IFN production, Vero cells, a line that does not produce IFN, were used in the same assay. Again, the V-deficient measles virus was a more potent inducer of cytopathic effect than wild-type virus. To-

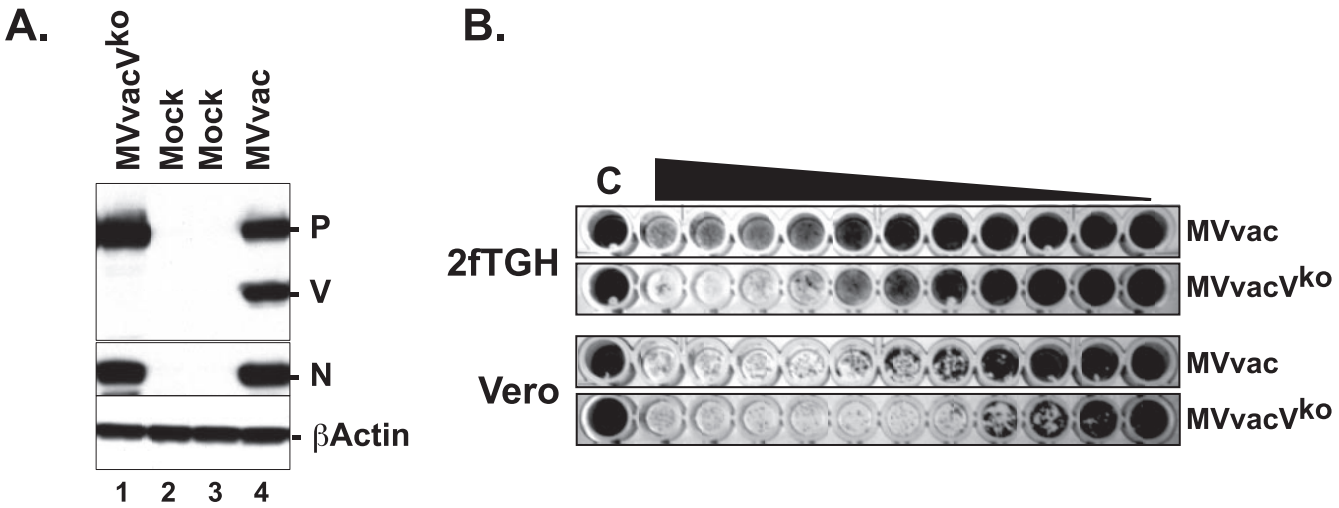

FIG. 5. Measles virus lacking V protein is more cytopathic. (A) Immunoblot analysis of the parental virus MVvac and its derivative, MVvacV^{ko}. HeLa cells were infected with parental virus MVvac (lane 4) and its derivative, MVvacV^{ko} (lane 1). Antisera against P/V proteins (upper panel) and against the N protein (middle panel) were used. Anti-β-actin (lower panel) was used as a loading control. Mock, uninfected cell control (lanes 2 and 3). (B) 2fTGH cells (top two panels) and Vero cells (bottom two panels) were plated in a 96-well dish and then infected with a serial 1:2 dilution of MVvac or MVvacV^{ko} virus in 50 μl of serum-free Dulbecco's modified Eagle's medium, beginning with 1×10^5 PFU in well 2. One hundred fifty microliters Dulbecco's modified Eagle's medium plus 5% serum was added at 2 h postinfection, and plates were incubated at 37°C for 72 h prior to fixation and staining with 3% methylene blue in 50% ethanol. C, control uninfected cells.

gether, these results provide genetic support for the concept that the measles virus V protein is a modulator of virus-induced cell death and are in agreement with prior studies of paramyxovirus V proteins.

Induction of cell death in response to virus infection is an effective antiviral immune response, as it not only prevents virus replication, but also can stimulate the clearance of infected cells by phagocytic cells and contribute to specific adaptive immunity. The ability to block virus-induced cell death would keep the cells alive during the course of virus infection, enhancing replication and enabling the spread of infection. Inactivation of proapoptotic signaling pathways would therefore be highly advantageous for virus replication and may be a key factor selecting for the evolution of viral p53/p73 family antagonists.

Protein interaction analysis implicated p53 and p73 as potential targets and demonstrated that measles V can interact with the conserved p53 family DNA binding domain. Mechanistic studies determined that the transcriptional activity of p73 is specifically inactivated by measles virus V protein. Measles virus V protein expression induces a delay in PARP cleavage in response to genotoxic stress, and suppression of p73-dependent transcription results in a substantial inhibition of steady-state PUMA expression in the measles virus V-containing cells, providing a plausible explanation for the antiapoptotic actions of measles virus V.

Enhanced cytopathicity was observed during infections with V-deficient measles virus. While many factors can contribute to the cytopathic effect assay, measles virus has been previously demonstrated to induce cytopathic effects via characteristic apoptotic pathways (6). Although previous evaluation of recombinant measles viruses did not uncover antiapoptotic effects attributable to V protein in cultured cells (33), V-deficient mutant viruses had altered growth and virulence properties in animal models (20, 29, 41). The exact reason for this discrepancy is not readily apparent from the literature but might reflect differences in methodology or cell lines used. A more trivial explanation could relate to the reported inability of the Edmonston tag strain V protein to antagonize IFN responses compared with other measles strains (23), possibly pointing toward loss-of-function mutations in the V protein (33). Notably, the increased cytopathicity of V-deficient measles virus appears to be less pronounced than that reported for V-deficient SV5 (10, 43, 44), and this could reflect the presence of measles virus C protein, which might also participate in modulation of host responses to infection. Further experimentation will be required to evaluate the effects of C protein in the assays for cell death carried out in the stable cell lines, as C protein expression has not been assessed. However, the coprecipitation analysis is strictly specific for V protein and the recombinant viruses retain C protein expression. Irrespective of potential C protein contributions, it is a common observation that genetic disruption of paramyxovirus V proteins results in viruses with enhanced cell death capacities, suggesting that avoidance of cell death is a general property of the paramyxovirus V protein.

Despite its ability to bind p53, no inhibition of p53-mediated gene regulation was observed in measles virus V-expressing cells. The underlying reason for this discrepancy has not been revealed by these studies, but this finding indicates specificity in measles virus V host interference. The results do not rule out the possibility of a functional physical association with p53 that does not influence transcriptional activity or coprecipitation of p53 via a bridging factor. As measles virus V protein interacts with the most highly conserved region shared by p53 and p73, the DNA binding domain, it would be reasonable to suspect the coprecipitation with p53 might be a result of molecular mimicry due to structural similarities between p73 and

p53 in this domain. Narrowing the precise mode of p73 inhibition will require further investigation, but the association with the DNA binding domain suggests that measles virus V might interfere with p73-promoter contacts or alter essential coactivator recruitment. Regardless of the mechanism, the observed specificity of viral antagonism is similar to that observed for viral p53 inhibitors from adenovirus (E1B 55K), SV40 (T antigen), and human papillomavirus (E6). While all of these viral oncoproteins are effective p53 inhibitors, none of them targets p73 (16). Together with our observation of specific viral p73 antagonism, these results support divergent functions of these two family members with respect to virus replication.

The V proteins from a number of paramyxovirus species have been demonstrated to prevent innate antiviral immunity by distinct mechanisms. These include targeting JAK-STAT signal transduction downstream of interferon signals (reviewed in reference 11), inhibition of IFN biosynthesis in response to virus infection (30), and suppression of cell death (10, 43, 44). While measles virus V binds to both p53 and p73 DNA binding domains, transcriptional activity of p73 is specifically blocked by expressing measles virus V cDNA, and this is reflected in the regulation of the proapoptotic target gene, PUMA. While the interference with p73 function represents only one participant in the regulation of virus-induced cell death that might explain the function of V protein in antiapoptosis, there are likely additional components that contribute to the phenotype. However, these findings suggest a novel aspect of the p73 pathway leading to PUMA expression as a determinant of innate antiviral immunity and provide the first description of p73 antagonism by a negative-strand RNA virus.

We thank Stu Aaronson and his laboratory members for helpful discussions during the initial exploration of p53 and members of the Horvath laboratory for critiques and advice.

This work was supported by NIH grants AI50707 to C.M.H. and AI63476 to R.C.

REFERENCES

1. **Andrejeva, J., K. S. Childs, D. F. Young, T. S. Carlos, N. Stock, S. Goodbourn, and R. E. Randall.** 2004. The V proteins of paramyxoviruses bind the IFN-inducible RNA helicase, mda-5, and inhibit its activation of the IFN-beta promoter. Proc. Natl. Acad. Sci. USA **101:**17264–17269.
2. **Chin, Y. E., M. Kitagawa, W. C. Su, Z. H. You, Y. Iwamoto, and X. Y. Fu.** 1996. Cell growth arrest and induction of cyclin-dependent kinase inhibitor p21 WAF1/CIP1 mediated by STAT1. Science **272:**719–722.
3. **Devaux, P., V. von Messling, W. Songsungthong, C. Springfeld, and R. Cattaneo.** Unpublished data.
4. **Didcock, L., D. F. Young, S. Goodbourn, and R. E. Randall.** 1999. Sendai virus and simian virus 5 block activation of interferon-responsive genes: importance for virus pathogenesis. J. Virol. **73:**3125–3133.
5. **Didcock, L., D. F. Young, S. Goodbourn, and R. E. Randall.** 1999. The V protein of simian virus 5 inhibits interferon signalling by targeting STAT1 for proteasome-mediated degradation. J. Virol. **73:**9928–9933.
6. **Esolen, L. M., S. W. Park, J. M. Hardwick, and D. E. Griffin.** 1995. Apoptosis as a cause of death in measles virus-infected cells. J. Virol. **69:**3955–3958.
7. **Flores, E. R., S. Sengupta, J. B. Miller, J. J. Newman, R. Bronson, D. Crowley, A. Yang, F. McKeon, and T. Jacks.** 2005. Tumor predisposition in mice mutant for p63 and p73: evidence for broader tumor suppressor functions for the p53 family. Cancer Cell **7:**363–373.
8. **Flores, E. R., K. Y. Tsai, D. Crowley, S. Sengupta, A. Yang, F. McKeon, and T. Jacks.** 2002. p63 and p73 are required for p53-dependent apoptosis in response to DNA damage. Nature **416:**560–564.
9. **Goodbourn, S., L. Didcock, and R. E. Randall.** 2000. Interferons: cell signalling, immune modulation, antiviral response and virus countermeasures. J. Gen. Virol. **81:10:**2341–2364.
10. **He, B., R. G. Paterson, N. Stock, J. E. Durbin, R. K. Durbin, S. Goodbourn, R. E. Randall, and R. A. Lamb.** 2002. Recovery of paramyxovirus simian virus 5 with a V protein lacking the conserved cysteine-rich domain: the multifunctional V protein blocks both interferon-beta induction and interferon signaling. Virology **303:**15–32.
11. **Horvath, C. M.** 2004. Silencing STATs: lessons from paramyxovirus interferon evasion. Cytokine Growth Factor Rev. **15:**117–127.
12. **Hummer, B. T., X.-L. Li, and B. A. Hassel.** 2001. Role for p53 in gene induction by double-stranded RNA. J. Virol. **75:**7774–7777.
13. **Kwak, J. C., P. P. Ongusaha, T. Ouchi, and S. W. Lee.** 2003. IFI16 as a negative regulator in the regulation of p53 and p21(Waf1). J. Biol. Chem. **278:**40899–40904.
14. **Lin, G. Y., and R. A. Lamb.** 2000. The paramyxovirus simian virus 5 V protein slows progression of the cell cycle. J. Virol. **74:**9152–9166.
15. **Lin, G. Y., R. G. Paterson, C. D. Richardson, and R. A. Lamb.** 1998. The V protein of the paramyxovirus SV5 interacts with damage-specific DNA binding protein. Virology **249:**189–200.
16. **Marin, M. C., C. A. Jost, M. S. Irwin, J. A. DeCaprio, D. Caput, and W. G. Kaelin, Jr.** 1998. Viral oncoproteins discriminate between p53 and the p53 homolog p73. Mol. Cell. Biol. **18:**6316–6324.
17. **Marques, J. T., D. Rebouillat, C. V. Ramana, J. Murakami, J. E. Hill, A. Gudkov, R. H. Silverman, G. R. Stark, and B. R. G. Williams.** 2005. Down-regulation of p53 by double-stranded RNA modulates the antiviral response. J. Virol. **79:**11105–11114.
18. **Melino, G., F. Bernassola, M. Ranalli, K. Yee, W. X. Zong, M. Corazzari, R. A. Knight, D. R. Green, C. Thompson, and K. H. Vousden.** 2004. p73 induces apoptosis via PUMA transactivation and Bax mitochondrial translocation. J. Biol. Chem. **279:**8076–8083.
19. **Moll, U. M., and N. Slade.** 2004. p63 and p73: roles in development and tumor formation. Mol. Cancer Res. **2:**371–386.
20. **Mrkic, B., B. Odermatt, M. A. Klein, M. A. Billeter, J. Pavlovic, and R. Cattaneo.** 2000. Lymphatic dissemination and comparative pathology of recombinant measles viruses in genetically modified mice. J. Virol. **74:**1364–1372.
21. **Murray, C. J., and A. D. Lopez.** 1997. Mortality by cause for eight regions of the world: Global Burden of Disease Study. Lancet **349:**1269–1276.
22. **Neil, J. C., E. R. Cameron, and E. W. Baxter.** 1997. p53 and tumour viruses: catching the guardian off-guard. Trends Microbiol. **5:**115–120.
23. **Ohno, S., N. Ono, M. Takeda, K. Takeuchi, and Y. Yanagi.** 2004. Dissection of measles virus V protein in relation to its ability to block alpha/beta interferon signal transduction. J. Gen. Virol. **85:**2991–2999.
24. **Ouchi, T., S. W. Lee, M. Ouchi, S. A. Aaronson, and C. M. Horvath.** 2000. Collaboration of signal transducer and activator of transcription 1 (STAT1) and BRCA1 in differential regulation of IFN-gamma target genes. Proc. Natl. Acad. Sci. USA **97:**5208–5213.
25. **Ouchi, T., A. N. Monteiro, A. August, S. A. Aaronson, and H. Hanafusa.** 1998. BRCA1 regulates p53-dependent gene expression. Proc. Natl. Acad. Sci. USA **95:**2302–2306.
26. **Palosaari, H., J.-P. Parisien, J. J. Rodriguez, C. M. Ulane, and C. M. Horvath.** 2003. STAT protein interference and suppression of cytokine signal transduction by measles virus V protein. J. Virol. **77:**7635–7644.
27. **Parisien, J.-P., J. F. Lau, and C. M. Horvath.** 2002. STAT2 acts as a host range determinant for species-specific paramyxovirus interferon antagonism and simian virus 5 replication. J. Virol. **76:**6435–6441.
28. **Park, M.-S., A. Garcia-Sastre, J. F. Cros, C. F. Basler, and P. Palese.** 2003. Newcastle disease virus V protein is a determinant of host range restriction. J. Virol. **77:**9522–9532.
29. **Patterson, J. B., D. Thomas, H. Lewicki, M. A. Billeter, and M. B. Oldstone.** 2000. V and C proteins of measles virus function as virulence factors in vivo. Virology **267:**80–89.
30. **Poole, E., B. He, R. A. Lamb, R. E. Randall, and S. Goodbourn.** 2002. The V proteins of simian virus 5 and other paramyxoviruses inhibit induction of interferon-beta. Virology **303:**33–46.
31. **Ronco, L. V., A. Y. Karpova, M. Vidal, and P. M. Howley.** 1998. Human papillomavirus 16 E6 oncoprotein binds to interferon regulatory factor-3 and inhibits its transcriptional activity. Genes Dev. **12:**2061–2072.
32. **Scheffner, M., B. A. Werness, J. M. Huibregtse, A. J. Levine, and P. M. Howley.** 1990. The E6 oncoprotein encoded by human papillomavirus types 16 and 18 promotes the degradation of p53. Cell **63:**1129–1136.
33. **Schneider, H., K. Kaelin, and M. A. Billeter.** 1997. Recombinant measles viruses defective for RNA editing and V protein synthesis are viable in cultured cells. Virology **227:**314–322.
34. **Sun, M., T. A. Rothermel, L. Shuman, J. A. Aligo, S. Xu, Y. Lin, R. A. Lamb, and B. He.** 2004. Conserved cysteine-rich domain of paramyxovirus simian virus 5 V protein plays an important role in blocking apoptosis. J. Virol. **78:**5068–5078.
35. **Takaoka, A., S. Hayakawa, H. Yanai, D. Stoiber, H. Negishi, H. Kikuchi, S. Sasaki, K. Imai, T. Shibue, K. Honda, and T. Taniguchi.** 2003. Integration of interferon-alpha/beta signalling to p53 responses in tumour suppression and antiviral defence. Nature **424:**516–523.
36. **Takeuchi, K., S. I. Kadota, M. Takeda, N. Miyajima, and K. Nagata.** 2003. Measles virus V protein blocks interferon (IFN)-alpha/beta but not IFN-gamma signaling by inhibiting STAT1 and STAT2 phosphorylation. FEBS Lett. **545:**177–182.
37. **Technau-Ihling, K., C. Ihling, J. Kromeier, and G. Brandner.** 2001. Influenza A virus infection of mice induces nuclear accumulation of the tumor suppressor protein p53 in the lung. Arch. Virol. **146:**1655–1666.

38. **Turpin, E., K. Luke, J. Jones, T. Tumpey, K. Konan, and S. Schultz-Cherry.** 2005. Influenza virus infection increases p53 activity: role of p53 in cell death and viral replication. J. Virol. **79:**8802–8811.
39. **Ulane, C. M., and C. M. Horvath.** 2002. Paramyxoviruses SV5 and HPIV2 assemble STAT protein ubiquitin ligase complexes from cellular components. Virology **304:**160–166.
40. **Ulane, C. M., A. Kentsis, C. D. Cruz, J.-P. Parisien, K. L. Schneider, and C. M. Horvath.** 2005. Composition and assembly of STAT-targeting ubiquitin ligase complexes: paramyxovirus V protein carboxyl terminus is an oligomerization domain. J. Virol. **79:**10180–10189.
41. **Valsamakis, A., H. Schneider, P. G. Auwaerter, H. Kaneshima, M. A. Billeter, and D. E. Griffin.** 1998. Recombinant measles viruses with mutations in the C, V, or F gene have altered growth phenotypes in vivo. J. Virol. **72:**7754–7761.
42. **Vassilev, L. T., B. T. Vu, B. Graves, D. Carvajal, F. Podlaski, Z. Filipovic, N. Kong, U. Kammlott, C. Lukacs, C. Klein, N. Fotouhi, and E. A. Liu.** 2004. In vivo activation of the p53 pathway by small-molecule antagonists of MDM2. Science **303:**844–848.
43. **Wansley, E. K., J. M. Grayson, and G. D. Parks.** 2003. Apoptosis induction and interferon signaling but not IFN-beta promoter induction by an SV5 P/V mutant are rescued by coinfection with wild-type SV5. Virology **316:**41–54.
44. **Wansley, E. K., and G. D. Parks.** 2002. Naturally occurring substitutions in the P/V gene convert the noncytopathic paramyxovirus simian virus 5 into a virus that induces alpha/beta interferon synthesis and cell death. J. Virol. **76:**10109–10121.
45. **Young, D. F., N. Chatziandreou, B. He, S. Goodbourn, R. A. Lamb, and R. E. Randall.** 2001. Single amino acid substitution in the V protein of simian virus 5 differentiates its ability to block interferon signaling in human and murine cells. J. Virol. **75:**3363–3370.
46. **Young, D. F., L. Didcock, S. Goodbourn, and R. E. Randall.** 2000. Paramyxoviridae use distinct virus-specific mechanisms to circumvent the interferon response. Virology **269:**383–390.
47. **Yuan, R., Q. Meng, H. Hu, I. D. Goldberg, E. M. Rosen, and S. Fan.** 2001. P53-independent downregulation of p73 in human cancer cells treated with adriamycin. Cancer Chemother. Pharmacol. **47:**161–169.