[CANCER RESEARCH 60, 4889–4893, September 1, 2000]

Ionizing Radiation-induced Apoptosis via Separate Pms2- and p53-dependent Pathways[1]

Ming Zeng, Latha Narayanan, Xiaoxin S. Xu, Tomas A. Prolla, R. Michael Liskay, and Peter M. Glazer[2]

Departments of Therapeutic Radiology and Genetics, Yale University School of Medicine, New Haven, Connecticut 06520-8040 [M. Z., L. N., X. S. X., P. M. G.]; Department of Molecular and Medical Genetics, Oregon Health Sciences University, Portland, Oregon 97201-3098 [R. M. L.]; and University of Wisconsin–Madison, Departments of Genetics and Medical Genetics, Madison, Wisconsin 53706 [T. A. P.]

ABSTRACT

The cytotoxicity of ionizing radiation (IR) has been associated with both the p53 pathway and with DNA mismatch repair (MMR). p53 mediates cell cycle arrest and apoptosis in response to X-ray damage, whereas the MMR complex is thought to recognize damaged bases and initiate a signal transduction pathway that can include phosphorylation of p53. To determine whether p53 and MMR mediate X-ray cytotoxicity via the same pathway, mice with targeted disruptions in either the *p53* gene or the MutL homologue MMR gene *Pms2* were interbred and primary fibroblasts were established from the progeny with genotypes of either wild type, p53 null, Pms2 null, or double null. Cells with either p53 or Pms2 separately disrupted showed reduced levels of apoptosis after IR in comparison with wild type, but the double null cells showed even lower levels, consistent with nonoverlapping roles for p53 and PMS2 in the X-ray response. In transformed cell lines established from the primary cells at early passage, similar differences in the apoptotic response to IR were seen, and clonogenic survival assays following low dose rate IR further showed that nullizygosity for Pms2 confers increased survival on cells in both wild-type and p53 null backgrounds. These results indicate that both p53 and MMR contribute to X-ray-induced apoptosis and that the role of MMR in the cytotoxicity of IR does not depend on p53.

INTRODUCTION

DNA MMR[3] deficiency has been linked to hereditary colon cancer and to an increasing number of sporadic cancers (1). The MMR pathway corrects bp mismatches and other DNA polymerase errors and serves to maintain genome integrity. However, MMR factors have also been implicated in the response of mammalian cells to DNA damage. On the basis of early work in bacteria suggesting a role for the MMR factors in response to alkylation damage (2), more recent work has established that mammalian cells deficient in MMR show tolerance to alkylating agents (3, 4). These observations have been extended to a variety of other DNA damaging agents, including several used clinically in cancer therapy, such as *cis*-platinum (5) and temozolomide (6).

We previously reported data suggesting a role for the MMR factors MSH2, MLH1, and PMS2 in the cytotoxicity of IR (7), with results showing a small but statistically significant increase in clonogenic survival after IR of MMR mutant cells compared with wild type. These studies were carried out in immortalized cell lines established from transgenic mice in which the *Msh2*, *Mlh1*, and *Pms2* genes were mutated by targeted disruption in mouse embryonic stem cells (7). On the basis of our results, we proposed a model in which the subset of radiation damage consisting of oxidized bases could be subject to the same MMR-mediated pathway of cytotoxicity as alkylated bases. However, several other studies using MMR-deficient human cancer-derived cell lines or immortalized Msh2-deficient mouse lines failed to find substantial or consistent differences in radiation response (8, 9), and so the finding has been subject to controversy. Recently, DeWeese *et al.* (10), focusing on cells from *Msh2* knockout mice, reproduced and extended our observations to show that at low dose rates the survival differences between wild-type and Msh2-deficient cells are even larger than the differences seen at high dose rates. In addition, Zhang *et al.* (11) found that IR-induced apoptosis was reduced in *Msh2*-nullizygous mouse embryos fibroblasts compared with wild type.

The mechanism by which the MMR complex may influence damage response is not yet clear. One hypothesis proposes that the MMR complex recognizes base damage and initiates a cycle of futile repair (12), leading to gaps and breaks that may ultimately signal apoptosis. It is also possible that the recognition of damage by the MMR complex directly initiates a signal transduction pathway, triggering apoptosis. Evidence supporting a central role for signal transduction in the MMR-mediated damage response includes a requirement for MLH1 function in *cis*-platinum induction of c-abl kinase activity and of p73 accumulation (13). In addition, the MSH2/MSH6 and the MLH1/PMS2 complexes were shown to be required for the phosphorylation of p53 at serines 15 and 392 following treatment of cells with alkylating agents (14). Consistent with this, in human colon cancer cell lines, an MLH1-dependent induction of p53 following IR was observed (9), and a MMR-deficient lymphoblastoid cell line showed reduced accumulation of p53 following temozolomide exposure (15). MMR-dependent induction of p53 was also seen in response to a variety of carcinogens (16).

The proposed role of p53 in the MMR-mediated pathway of damage response is important for a variety of reasons. p53 is mutated in a large number of human cancers (17) and has roles in cell cycle regulation, transcription, and apoptosis (18). Many lines of evidence also implicate p53 as a central factor in the cellular response to IR, leading to cell cycle checkpoint activation and apoptosis (19). Recent work using a series of Chinese hamster fibroblast and human lymphoblastoid cell lines suggests, however, that the MMR-mediated apoptotic response to the alkylating agent *N*-methyl-*N′*-nitro-*N*-nitrosoguanidine may be independent of p53 (20), raising questions regarding the functional importance of MMR-dependent signaling of alkylation damage through p53 phosphorylation.

To further examine the potential roles of MMR and p53 and their putative interdependence in the cellular response to IR, we have interbred mice carrying targeted disruptions at the *Pms2* (21, 22) and *p53* loci (23) to generate primary embryo fibroblasts with defined genotypes at these loci, including wild type, p53 null, Pms2 null, and double null. We reasoned that such cells, being genetically well defined, would constitute a more meaningful test system than the series of cancer-derived cell lines that are frequently used, because the latter cells are likely to contain a number of other accumulated mutations and abnormalities that could influence radiation response. Using these cells, we report here that apoptosis of primary fibroblasts after IR is reduced by separate inactivation of either p53 or Pms2 and that inactivation of both together results in even further reduction in

Received 3/27/00; accepted 6/30/00.

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.

[1] Supported by NIH Grant ES05775.

[2] To whom requests for reprints should be addressed, at Department of Therapeutic Radiology, Yale University School of Medicine, P.O. Box 208040, New Haven, CT 06520-8040. Phone: (203) 737-2788; Fax: (203) 737-2630; E-mail: peter.glazer@yale.edu.

[3] The abbreviations used are: MMR, mismatch repair; IR, ionizing radiation.

Downloaded from cancerres.aacrjournals.org on September 20, 2021. © 2000 American Association for Cancer Research.

the apoptotic response, suggesting nonoverlapping roles for these factors in response to IR. In addition, experiments using cell lines established from these primary cells at early passage revealed increased clonogenic survival after IR in cells nullizygous for Pms2, regardless of p53 status, further indicating that MMR-mediated cytotoxicity does not depend on p53.

MATERIALS AND METHODS

Mice. Knockout mice carrying targeted disruptions of the *p53* loci have been described previously (23) and were provided to one of us (T. A. P.) in the laboratory of Allan Bradley (Baylor College of Medicine, Houston, TX). Mice with disruption of the *Pms2* loci have been described previously (21). Mice heterozygous for Pms2 were bred to either wild-type or p53 null mice, and the resulting progeny were interbred to generate two sets of littermates: Pms2 null and wild type in a p53 wild-type background, or Pms2 null and wild type in a p53 null background. The genotypes were confirmed by PCR amplification of the *Pms2* and *p53* loci to detect the presence or absence of the targeted disruption, as described previously (21, 23).

Cells. Mouse primary embryo fibroblasts were established as described previously (7) and were cryopreserved at passage 3. Experiments were conducted on cells immediately after recovery and expansion from frozen storage. Transformation of the primary cells was carried out by transfection with E1A and Ha-Ras expression vectors, as described (24). Cells at 60% confluence were transfected with GenePorter cationic lipid reagent (Gene Therapy Systems, San Diego, CA) according to the manufacturer's instructions. The following day, cells were re-seeded at serial dilutions into selective media containing 50 μg/ml hygromycin. Successfully transfected clones were isolated 12–14 days later as foci of morphologically transformed cells, and selected colonies were expanded and analyzed by Western blot for expression of PMS2 and p53. Established cell lines were grown in DMEM supplemented with 10% FCS and 2× amino acids, 2× vitamins, 2× nonessential amino acids, penicillin, and streptomycin (Life Technologies, Inc., Bethesda, MD).

Western Blot Analysis. Total cellular extracts were prepared by incubating cells in a lysis buffer [20 mM Tris/HCl (pH 8.0), 137 mM NaCl, 10% glycerol, 1% NP40, 10 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride, and 1 mM benzamidine] for 30 min. Extract protein (30 μg), measured by using the Bradford assay (Bio-Rad), was separated by SDS-PAGE, transferred to nitrocellulose, probed with either anti-PMS2 (PMS2/AB-1, mouse monoclonal, catalogue no. NA30; Oncogene Research Products, Cambridge, MA) or anti-p53 (mouse monoclonal, catalogue no. 13–4100; Zymed Laboratories, San Francisco, CA) antibodies, both at 1:1000 dilutions, and visualized by enhanced chemiluminescence (ECL kit; Amersham Corp.).

IR Exposure. For high dose rate treatments, cells were irradiated using a Cs-137 irradiator at a dose rate of 225 cGy/min in growth medium, as described (7). For low dose rate exposures, a 9.7-mCi Americium source was set up within a cell culture incubator. The source was placed in a $20 \times 20 \times 10\text{-cm}^3$ polystyrene phantom with a 10.2-cm diameter centered hole. The cell culture dishes were positioned within the hole for irradiation. Dosimetry was determined as described previously (25). The dose rate was determined to be between 16–27 cGy/h, depending on the position of the dishes. Control, unirradiated flasks were incubated under the same conditions in the absence of the source. Following irradiation, cultures were incubated under normal growth conditions.

Measurement of Apoptosis. Cells were irradiated as above, and apoptosis was assayed at various times by harvesting the adherent cells and labeling DNA ends using the ApoTag kit (Intergen Co.), according to the manufacturer's instructions. Fluorescently tagged cells were quantified using fluorescence-activated cell-sorting analysis, using a FACScan flow cytometer (Becton Dickinson, San Jose, CA).

Clonogenic Survival Assays. The transformed mouse cell lines were seeded into 100-mm dishes by serial dilutions and were exposed to IR, as above. Surviving colonies were fixed and stained with crystal violet for visualization after 14 days in culture (7). For each experiment, three replicate dishes were examined at each dose, and only those colonies containing ~50 or more cells were scored as surviving clones. After normalization for the plating efficiencies of the cell lines, the fractional survival was determined at each dose.

RESULTS

Generation of p53- and Pms2-deficient Mice and Primary Cells and Cell Lines. To obtain primary cells with defined genotypes at the *p53* and *Pms2* loci, mice heterozygous for Pms2 were bred to either wild-type or p53 null animals, and progeny animals were intercrossed to generate mice either wild type or null for Pms2 in a p53 wild-type background or wild type and null for Pms2 in the setting of p53 nullizygosity. We then established primary mouse fibroblasts as described before (7). Early passages of these four kinds of primary fibroblasts were used in subsequent studies.

To study clonogenic survival, established cell lines are necessary because of the poor cloning efficiency of primary cells. We used a combination of adenovirus E1A and Ha-Ras to transform the cells at early passage because mouse embryo fibroblasts transformed by these genes have been shown to retain p53-dependent apoptotic responses (24). Transfected clones growing in selective media were identified and expanded. In selected clones, we confirmed the expected genotypes by determining expression of p53 and PMS2 via immunoblot analysis (Fig. 1), and the cell lines used are listed in Table 1.

Pms2- and p53-dependent Apoptosis in Primary Cells. To study the role of p53 and Pms2 in the cellular response to ionizing irradiation, we used subconfluent primary fibroblast cultures to examine X-ray-induced apoptosis after a dose of 20 Gy. The advantage of using primary cells of defined genotype is that accumulated mutations and abnormalities in other factors that could affect the apoptotic response are unlikely to be present. To measure apoptosis, DNA fragmentation was assayed using a fluorescent end-labeling technique. Initially, a time course was carried out after IR exposure using the wild-type cells. Over the course of 5 days, progressive deterioration of the monolayer was observed, with detachment and fragmentation of dead cells. It was judged that reliable measurements of apoptosis would be limited to the first 2 days, during which the cell culture monolayer remained mostly intact. Over this period, it was determined that the peak induction of apoptosis as measured by DNA fragmentation of adherent cells was seen at 18 h (Fig. 2*A*). Subsequent assays were conducted 18 h after IR, and the relative percentage of apoptosis in the wild-type, p53 null, Pms2 null, and double null cells was scored (Fig. 2*B*). In comparison with the pattern observed in the wild-type primary fibroblasts, the apoptotic responses of the p53 null and the Pms2 null cells were diminished. However, the double null cells showed an even greater reduction in apoptotic response than the singly null cells. These results indicate that p53 and Pms2 both play a role in IR-induced apoptosis and that they act in nonoverlapping pathways because the effects of p53 knockout and Pms2 knockout are additive.

Apoptosis in Established Cell Lines. To confirm these results, we examined the response to IR in cell lines established from the primary

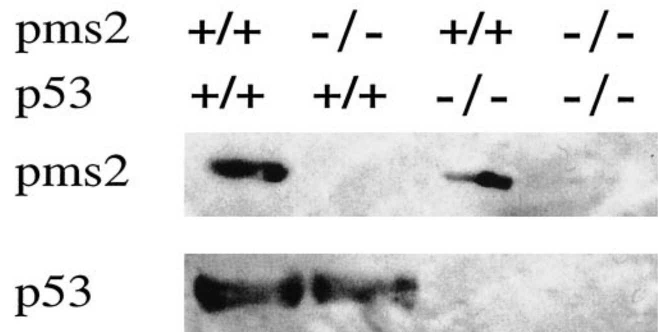

Fig. 1. Western blot analysis of p53 and PMS2 expression to confirm genotypes of selected transformed cell lines isolated after E1A/Ha-Ras transfection of mouse primary cells. The details of immunoblotting are as described in "Materials and Methods."

Downloaded from cancerres.aacrjournals.org on September 20, 2021. © 2000 American Association for Cancer Research.

Table 1 *Fibroblast cell lines established from knockout mice*

Cell line	Genotype
CL7	*p53* +/+, *Pms2* +/+
CL11	*p53* +/+, *Pms2* −/−
TF6	*p53* −/−, *Pms2* +/+
TF11	*p53* −/−, *Pms2* −/−

cells by transformation with E1A and Ha-Ras (Table 1). In these experiments, the 40-h time point was chosen for analysis (time course data not shown). As with the primary cells, the wild-type transformed cells showed the highest degree of apoptosis (Fig. 3). The Pms2 nulls and the p53 nulls showed reduced levels of apoptosis, and the double null cells exhibited the lowest degree of apoptosis. Again, these data show that Pms2 knockout in the setting of p53 nullizygosity further reduces the IR-induced apoptotic response in comparison with the p53 null cells alone, indicating that Pms2 mediates additional apoptosis via a pathway independent of p53.

Radioresistance of Pms2 Nullizygous Cells by Clonogenic Survival Is Independent of p53 Status. In previous work, we reported that cell lines deficient in either Pms2, Msh2, or Mlh1 show increased clonogenic survival in response to IR. To determine whether this effect of MMR function on X-ray survival depends on p53, we carried out pairwise comparisons of clonogenic survival among transformed cells of defined genotype: C7 cells (Pms2 +/+; p53 +/+) *versus* the C11 cells (Pms2 −/−; p53 +/+) and TF6 cells (Pms2 +/+; p53 −/−) *versus* TF11 cells (Pms2 −/−; p53−/−; Table 1 and Fig. 4). Because DeWeese *et al.* (10) had made the observation that cells deficient in Msh2 show an even more pronounced difference in radioresistance on low dose rate as opposed to high dose rate irradiation, we carried out irradiations at both high and low dose rates. Fig. 4 shows the results from the low dose rate studies. The comparison of the C7 and C11 cells in the p53 wild-type background shows that the Pms2 null cells demonstrate increased survival after IR. In the case of the p53 null pair, there is increased survival in the Pms2-deficient line (TF11), indicating that there is an effect of PMS2 on clonogenic survival that does not depend on p53. At high dose rates, the Pms2-deficient cells also showed better survival compared with the Pms2 wild-type cells regardless of p53 status, although the differences were smaller than those seen at low dose rates (data not shown). Hence, the survival data provide additional evidence for the p53 independence of the Pms2-mediated response.

DISCUSSION

In this study, we have investigated the role of p53 in the MMR-mediated response of cells to IR. We have examined IR-induced apoptosis in primary fibroblasts established from transgenic mice carrying targeted disruptions of p53 and/or the MutL homologue MMR gene *Pms2*. Cells with four possible genetic backgrounds were compared in terms of IR-induced apoptosis. We found that deficiencies in either *p53* or *Pms2* genes are associated with reduced levels of IR-induced apoptosis compared with the wild type, consistent with roles for both factors in the cellular response to IR. In cells deficient in both p53 and Pms2 we observed even lower levels of apoptosis, indicating that MMR and p53 mediate IR-induced apoptosis via separate and apparently additive pathways.

Using genetically defined transformed cell lines derived from the mouse primary cells, we again found that cells nullizygous for either Pms2 or p53 show lower levels of apoptosis than do the wild type and that the apoptotic response is even further reduced in the doubly nullizygous cell line. Extending these results to an examination of clonogenic survival, it was found that lack of Pms2 renders cells more resistant to IR regardless of p53 status.

Our results indicate that the MMR-mediated apoptotic and cytotoxic response to IR does not depend on p53, consistent with a recent report that the MMR-mediated apoptotic response to *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine is not dependent on p53 (20). Nevertheless, it has been established that p53 phosphorylation is affected by certain MMR factors (14), and so there does seem to be some cross-talk between the MMR pathway and the p53 pathway. Although it is possible that some small degree of IR-induced apoptosis depends on this cross-talk, our results suggest that the majority of the detectable IR-induced, MMR-dependent apoptosis and cytotoxicity is independent of it. More likely, MMR-mediated signaling through p53 may play a role in other cellular responses, such as cell cycle checkpoint regulation.

On the other hand, a recent study in Msh2- and p53-deficient mice and mouse cells suggested that temozolomide-induced apoptosis me-

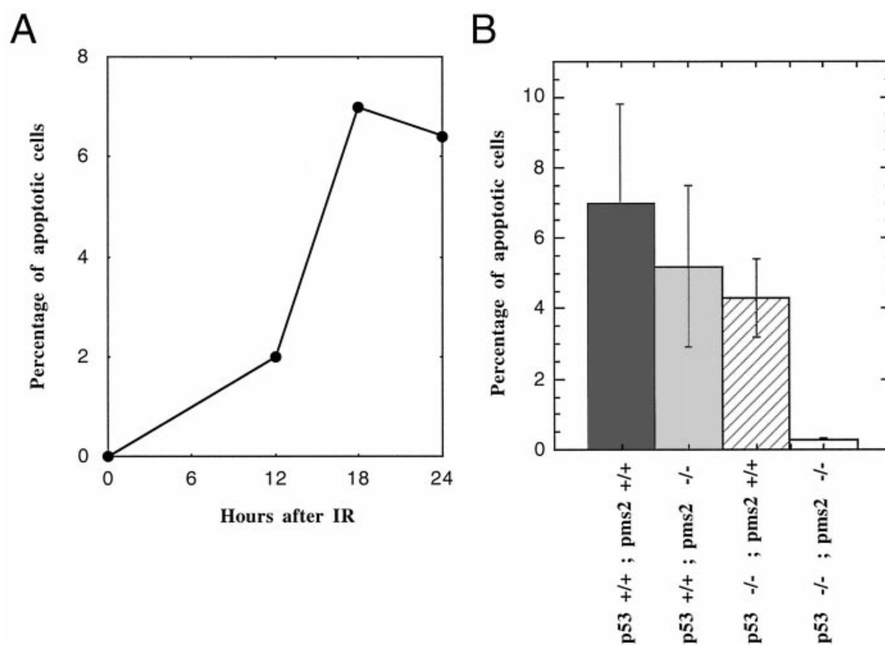

Fig. 2. Effect of deficiency of p53, Pms2, or both on apoptosis in primary mouse fibroblasts after treatment with IR. p53 and Pms2 transgenic mice were interbred to generate animals with genotypes either wild type, p53 null, Pms2 null, or double null, as a source of primary fibroblasts. Primary cells at passage 3 to 5 were irradiated with 20 Gy of IR, and apoptosis was measured by DNA fragmentation. *A*, time course of apoptosis observed in adherent wild-type cells following 20 Gy of IR. *B*, apoptosis in wild-type, p53 null, Pms2 null, or double null primary cells at 18 h after IR. Apoptotic cells were quantified by cell-sorting analysis. Each value represents the mean +/− SD of three separate determinations.

Downloaded from cancerres.aacrjournals.org on September 20, 2021. © 2000 American Association for Cancer Research.

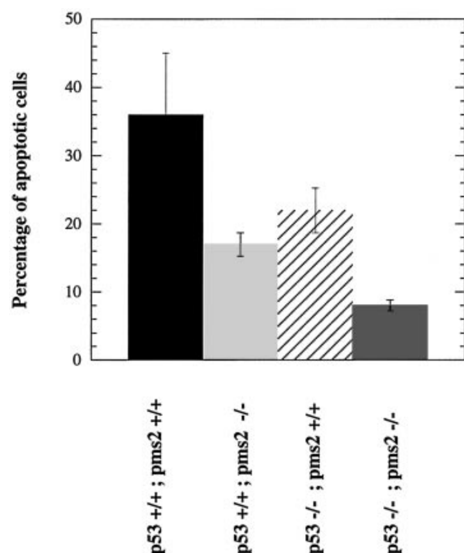

Fig. 3. Effect of p53 and Pms2 deficiencies on the frequency of apoptotic cells in E1A/Ha-Ras-transformed mouse fibroblasts after treatment with IR. Transformed mouse fibroblasts were established by cotransfection of E1A and Ha-Ras genes into the primary fibroblasts derived from wild-type, p53 null, Pms2 null, or double null animals. Apoptotic assays were performed 40 h after treatment with 20 Gy of IR, as shown in Fig. 2.

diated by Msh2 may proceed via both p53-dependent and -independent pathways (26). In addition, Wu *et al.* (16) reported that the induction of p53 in response to selected carcinogens is dependent on functional MMR and that the MMR-dependent apoptotic response to the chemicals was mediated through both p53-dependent and p53-independent pathways. Whether these results reflect inherent differences between cellular responses to IR *versus* various chemicals or to differences in the sensitivities of the assays remains to be determined. However, the observation of p53-independent, MMR-mediated pathways in some of the above studies is consistent with our findings and those of Hickman and Samson (20).

Examination of the clonogenic survival curves clearly indicates that most of the cytotoxicity of IR is actually independent of PMS2. IR generates a large number of lesions in DNA, including double-strand breaks, single-strand breaks, and a wide variety of base and sugar damage. It is likely that cell death from strand breaks is independent of MMR. Rather, we have hypothesized that it is the IR-induced base damage, or at least some subset of it, that is subject to MMR recognition (7). On the basis of the emerging model for the alkylation damage response pathway (13), we propose that this recognition initiates a signal transduction pathway that leads to apoptosis. The results presented here suggest that this pathway does not require p53, although MMR recognition of base damage may signal to p53 for other purposes. Aside from p53, recent work suggests that MMR-associated signaling involves a number of other factors, including c-*abl* and p73 (13). p73 is a homologue of p53, and one possibility is that MMR signals apoptosis following IR via a p73-dependent pathway. Such a role for p73 in the case of *cis*-platinum exposure was proposed (13).

The role of the MMR complex in the recognition and processing of oxidatively damaged bases, as arise from exposure to IR, has been suggested by the results of several studies. For example, Ni *et al.* (27) observed binding of MSH2/MSH6 complexes to DNA containing 8-oxo-guanine, and DeWeese *et al.* (10) reported increased accumulation of 8-oxo-guanine in Msh2-deficient mouse cells. Two studies of mutagenesis in yeast also indicated a role for MMR in mutagenesis related to oxidative base damage (27, 28).

The results reported here, furthermore, confirm our previous study of the role of MMR in the cytotoxicity of IR (7). In that study, several sets of wild-type and MMR-deficient mouse cell lines were compared for clonogenic survival in response to IR. Cells deficient in either Msh2, Mlh1, or Pms2 all showed increased survival relative to the matched wild type. The present work was performed on an independently derived set of mouse primary cells and cell lines and so adds additional evidence to support the basic observation.

Previous studies of MMR-associated apoptosis have identified MSH2 and MLH1 as key mediators of the process. For example, Msh2-deficient cells exhibited reduced apoptosis after IR in two studies (10, 11). In one study, simple overexpression of either MSH2 and MLH1, but not PMS2, MSH3, or MSH6, induced apoptosis in human cells (11). These latter observations suggested a special role for the factors MSH2 and MLH1 in the apoptotic response, raising questions as to the particular role of PMS2 in induced apoptosis. The results presented here directly demonstrate that PMS2 plays a role in damage-induced apoptosis, suggesting that formation and normal functioning of the MutSα (MSH2/MSH6) and MutLα (MLH1/PMS2) complexes are required for MMR-dependent, IR-induced apoptosis.

In addition, work by DeWeese *et al.* (10) implicated MSH2 in an exaggerated response to IR that is delivered at low dose rate. The work presented here shows that PMS2 also plays a role in the differential effects of low dose rate IR, suggesting, as above, that the effect of the low dose rate IR is mediated via recognition and processing by the MutSa and MutLa complexes and not simply by MSH2 or MLH1 alone. Whereas the data reported here show that the low dose rate effect does not depend on p53, the question remains as to the why there is a larger survival difference between MMR-proficient and -deficient cells at low dose rates. One possibility is that the lesion equilibrium, determined by the rate of radiation damage *versus* the rate of repair of the various types of lesions, is different at low as opposed to high dose rates, perhaps enhancing the effects of the subset of base damage that is subject to MMR recognition.

Although the work presented here, along with the several other studies noted above, establish that MMR can trigger apoptosis in a p53-independent pathway, our results also support the well established concept that p53 can mediate apoptosis following IR (29). Interestingly, however, the magnitude of the effect of Pms2 nullizy-

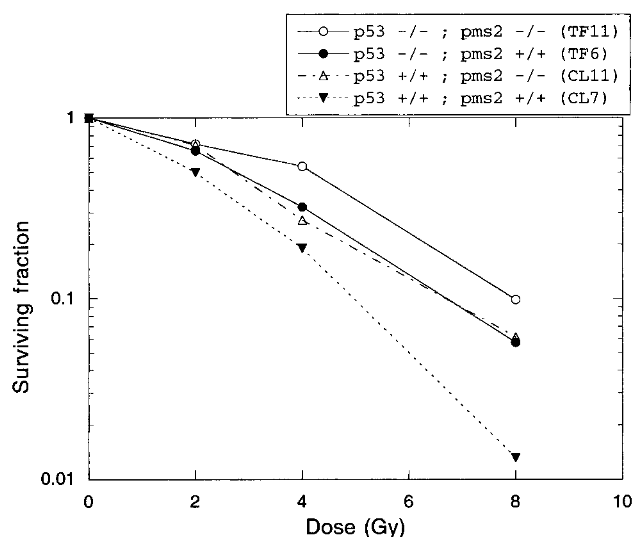

Fig. 4. Influence of Pms2 on the cytotoxicity of IR in cells either wild type or null for p53. The indicated doses of IR were delivered at a low dose rate (16 cGy/h for dose points 2 Gy and 4 Gy and 27 cGy/h for the 8-Gy dose point), and colony formation was determined. The curves indicate clonogenic survival of p53 wild-type cells that are either wild type (CL7) or null (CL11) for Pms2 and of p53 nullizygous cells that are either wild type (TF6) or nullizygous (TF11) for Pms2.

Downloaded from cancerres.aacrjournals.org on September 20, 2021. © 2000 American Association for Cancer Research.

gosity on apoptosis in our data were in the same range as that of p53 nullizygosity. Because evidence is emerging that many sporadic cancers, as well hereditary cancers, have deficiencies in MMR, these results serve to highlight the clinical importance of the MMR phenotype of human cancers.

ACKNOWLEDGMENTS

We thank J. A. Fritzell, J. Yuan, P. Bongiorni, R. Nath, A. Bradley, L. Cabral, R. Franklin, and S. J. Baserga for their help.

REFERENCES

1. Kolodner, R. Biochemistry and genetics of eukaryotic mismatch repair. Genes Dev., *10:* 1433–1442, 1996.
2. Karran, P., and Marinus, M. G. Mismatch correction at O^6-methylguanine residues in *E. coli* DNA. Nature, *296:* 868–869, 1982.
3. Branch, P., Aquilina, G., Bignami, M., and Karran, P. Defective mismatch binding and a mutator phenotype in cells tolerant to DNA damage. Nature (Lond.), *362:* 652–654, 1993.
4. Kat, A., Thilly, W. G., Fang, W. H., Longley, M. J., Li, G. M., and Modrich, P. An alkylation-tolerant, mutator human cell line is deficient in strand-specific mismatch repair. Proc. Natl. Acad. Sci. USA, *90:* 6424–6428, 1993.
5. Fink, D., Nebel, S., Aebi, S., Zheng, H., Cenni, B., Nehmé, A., Christen, R. D., and Howell, S. B. The role of DNA mismatch repair in platinum drug resistance. Cancer Res., *56:* 4881–4886, 1996.
6. Liu, L., Markowitz, S., and Gerson, S. L. Mismatch repair mutations override alkyltransferase in conferring resistance to temozolomide but not to 1,3-Bis(2-chloroethyl)nitrosourea. Cancer Res., *56:* 5375–5379, 1996.
7. Fritzell, J. A., Narayanan, L., Baker, S. M., Bronner, C. E., Andrew, S. E., Prolla, T. A., Bradley, A., Jirik, F. R., Liskay, R. M., and Glazer, P. M. Role of DNA mismatch repair in the cytotoxicity of ionizing radiation. Cancer Res., *57:* 5143–5147, 1997.
8. Reitmair, A. H., Risley, R., Bristow, R. G., Wilson, T., Ganesh, A., Jang, A., Peacock, J., Benchimol, S., Hill, R. P., Mak, T. W., Fishel, R., and Meuth, M. Mutator phenotype in *Msh2*-deficient murine embryonic fibroblasts. Cancer Res., *57:* 3765–3771, 1997.
9. Davis, T. W., Wilson-Van Patten, C., Meyers, M., Kunugi, K. A., Cuthill, S., Reznikoff, C., Garces, C., Boland, C. R., Kinsella, T. J., Fishel, R., and Boothman, D. A. Defective expression of the DNA mismatch repair protein, MLH1, alters G_2-M cell cycle checkpoint arrest following ionizing radiation. Cancer Res., *58:* 767–778, 1998.
10. DeWeese, T. L., Shipman, J. M., Larrier, N. A., Buckley, N. M., Kidd, L. R., Groopman, J. D., Cutler, R. G., te Riele, H., and Nelson, W. G. Mouse embryonic stem cells carrying one or two defective Msh2 alleles respond abnormally to oxidative stress inflicted by low-level radiation. Proc. Natl. Acad. Sci. USA, *95:* 11915–11920, 1998.
11. Zhang, H., Richards, B., Wilson, T., Lloyd, M., Cranston, A., Thorburn, A., Fishel, R., and Meuth, M. Apoptosis induced by overexpression of *hMSH2* or *hMLH1*. Cancer Res., *59:* 3021–3027, 1999.
12. Vaisman, A., Varchenko, M., Umar, A., Kunkel, T. A., Risinger, J. I., Barrett, J. C., Hamilton, T. C., and Chaney, S. G. The role of hMLH1, hMSH3, and hMSH6 defects in cisplatin and oxaliplatin resistance: correlation with replicative bypass of platinum-DNA adducts. Cancer Res., *58:* 3579–3585, 1998.
13. Gong, J. G., Costanzo, A., Yang, H. Q., Melino, G., Kaelin, W. G., Jr., Levrero, M., and Wang, J. Y. The tyrosine kinase c-Abl regulates p73 in apoptotic response to cisplatin-induced DNA damage. Nature (Lond.), *399:* 806–809, 1999.
14. Duckett, D. R., Bronstein, S. M., Taya, Y., and Modrich, P. hMutS α- and hMutL α-dependent phosphorylation of p53 in response to DNA methylator damage. Proc. Natl. Acad. Sci. USA, *96:* 12384–12388, 1999.
15. D'Atri, S., Tentori, L., Lacal, P. M., Graziani, G., Pagani, E., Benincasa, E., Zambruno, G., Bonmassar, E., and Jiricny, J. Involvement of the mismatch repair system in temozolomide-induced apoptosis. Mol. Pharmacol., *54:* 334–341, 1998.
16. Wu, J. X., Gu, L. Y., Wang, H. X., Geacintov, N. E., and Li, G. M. Mismatch repair processing of carcinogen-DNA adducts triggers apoptosis. Mol. Cell. Biol., *19:* 8292–8301, 1999.
17. Hollstein, M., Sidransky, D., Vogelstein, B., and Harris, C. C. p53 mutations in human cancers. Science (Washington DC), *253:* 49–53, 1991.
18. Levine, A. J. p53, the cellular gatekeeper for growth and division. Cell, *88:* 323–331, 1997.
19. Giaccia, A. J., and Kastan, M. B. The complexity of p53 modulation: emerging patterns from divergent signals. Genes Dev., *12:* 2973–2983, 1998.
20. Hickman, M. J., and Samson, L. D. Role of DNA mismatch repair and p53 in signaling induction of apoptosis by alkylating agents. Proc. Natl. Acad. Sci. USA, *96:* 10764–10769, 1999.
21. Baker, S. M., Bronner, C. E., Zhang, L., Plug, A. W., Robatzek, M., Warren, G., Elliott, E. A., Yu, J., Ashley, T., Arnheim, N., Flavell, R. A., and Liskay, R. M. Male mice defective in the DNA mismatch repair gene PMS2 exhibit abnormal chromosome synapsis in meiosis. Cell, *82:* 309–319, 1995.
22. Narayanan, L., Fritzell, J. A., Baker, S. M., Liskay, R. M., and Glazer, P. M. Elevated levels of mutation in multiple tissues of mice deficient in the DNA mismatch repair gene, *Pms2*. Proc. Natl. Acad. Sci. USA, *94:* 3122–3127, 1997.
23. Donehower, L. A., Harvey, M., Slagle, B. L., McArthur, M. J., Montgomery, C. A., Jr., Butel, J. S., and Bradley, A. Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. Nature (Lond.), *356:* 215–221, 1992.
24. Graeber, T. G., Osmanian, C., Jacks, T., Housman, D. E., Koch, C. J., Lowe, S. W., and Giaccia, A. J. Hypoxia-mediated selection of cells with diminished apoptotic potential in solid tumours. Nature (Lond.), *379:* 88–91, 1996.
25. Nath, R., and Gray, L. Dosimetry studies on prototype 241Am sources for brachytherapy. Int. J. Radiat. Oncol. Biol. Phys., *13:* 897–905, 1987.
26. Toft, N. J., Winton, D. J., Kelly, J., Howard, L. A., Dekker, M., te Riele, H., Arends, M. J., Wyllie, A. H., Margison, G. P., and Clarke, A. R. Msh2 status modulates both apoptosis and mutation frequency in the murine small intestine. Proc. Natl. Acad. Sci. USA, *96:* 3911–3915, 1999.
27. Ni, T. T., Marsischky, G. T., and Kolodner, R. D. MSH2 and MSH6 are required for removal of adenine misincorporated opposite 8-oxo-guanine in *S. cerevisiae*. Mol. Cell. , *4:* 439–444, 1999.
28. Earley, M. C., and Crouse, G. F. The role of mismatch repair in the prevention of base pair mutations in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA, *95:* 15487–15491, 1998.
29. Lowe, S. W., Schmitt, E. M., Smith, S. W., Osborne, B. A., and Jacks, T. p53 is required for radiation-induced apoptosis in mouse thymocytes. Nature (Lond.), *362:* 847–849, 1993.

Downloaded from cancerres.aacrjournals.org on September 20, 2021. © 2000 American Association for Cancer Research.

Cancer Research
The Journal of Cancer Research (1916–1930) | The American Journal of Cancer (1931–1940)

AACR American Association for Cancer Research

Ionizing Radiation-induced Apoptosis via Separate Pms2- and p53-dependent Pathways

Ming Zeng, Latha Narayanan, Xiaoxin S. Xu, et al.

Cancer Res 2000;60:4889-4893.

Updated version	Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/60/17/4889

Cited articles	This article cites 29 articles, 19 of which you can access for free at: http://cancerres.aacrjournals.org/content/60/17/4889.full#ref-list-1
Citing articles	This article has been cited by 10 HighWire-hosted articles. Access the articles at: http://cancerres.aacrjournals.org/content/60/17/4889.full#related-urls

E-mail alerts	Sign up to receive free email-alerts related to this article or journal.
Reprints and Subscriptions	To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.
Permissions	To request permission to re-use all or part of this article, use this link http://cancerres.aacrjournals.org/content/60/17/4889. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.

Downloaded from cancerres.aacrjournals.org on September 20, 2021. © 2000 American Association for Cancer Research.