DNA Mismatch Repair Initiates 6-Thioguanine – Induced Autophagy through p53 Activation in Human Tumor Cells

Xuehuo Zeng, Tao Yan, Jane E. Schupp, Yuji Seo, and Timothy J. Kinsella

Abstract **Purpose:** We investigate the roles of DNA mismatch repair (MMR) and p53 in mediating the induction of autophagy in human tumor cells after exposure to 6-thioguanine (6-TG), a chemotherapy drug recognized by MMR. We also examine how activation of autophagy affects apoptosis (type I cell death) after MMR processing of 6-TG.

Experimental Design: Using isogenic pairs of $MLH1^-$/$MLH1^+$ human colorectal cancer cells (HCT116) and $MSH2^-$/$MSH2^+$ human endometrial cancer cells (HEC59), we initially measure activation of autophagy for up to 3 days after 6-TG treatment using LC3, a specific marker of autophagy. We then assess the role of p53 in autophagic signaling of 6-TG MMR processing using both pifithrin-α cotreatment to chemically inhibit p53 transcription and small hairpin RNA inhibition of p53 expression. Finally, we use Atg5 small hairpin RNA inhibition of autophagy to assess the effect on apoptosis after MMR processing of 6-TG.

Results: We find that MMR is required for mediating autophagy in response to 6-TG treatment in these human tumor cells. We also show that p53 plays an essential role in signaling from MMR to the autophagic pathway. Finally, our results indicate that 6-TG – induced autophagy inhibits apoptosis after MMR processing of 6-TG.

Conclusions: These data suggest a novel function of MMR in mediating autophagy after a chemical (6-TG) DNA mismatch damage through p53 activation. The resulting autophagy inhibits apoptosis after MMR processing of 6-TG.

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The primary role of postreplicative mismatch repair (MMR) is to eliminate DNA polymerase errors, such as base-base mismatches and insertion-deletion loops from the newly synthesized strand after DNA replication in organisms from bacteria to mammals (1). DNA mismatches are recognized and initially bound by either MutSα, a heterodimer of MSH2 and MSH6, or MutSβ, a heterodimer of MSH2 and MSH3. After the interaction with a second complex, MutLα (a heterodimer of MLH1 and PMS2), the excision repair process is initiated (2). The MMR system is critical for maintaining the overall integrity of the genome.

Defects in MMR are associated with an increased risk of cancer, as cells deficient in MMR have a "mutator phenotype" in which the rate of spontaneous mutation is greatly elevated (3, 4). MMR deficiency is correlated with human hereditary nonpolyposis colorectal cancer and a small percentage (up to 15%) of several common sporadic tumors (3). Tumors in human hereditary nonpolyposis colorectal cancer patients result from mutations in the *MLH1* and *MSH2* genes in ~80% to 85% of cancers, whereas epigenetic silencing (methylation) of the *MLH1* and *MSH2* promoters are found in a majority of sporadic tumors (5, 6).

MMR deficiency also renders cells resistant to several classes of chemotherapy agents, such as the methylating agent *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (7), the platinum-based chemotherapy drug cisplatin (8), and the antimetabolite 6-thioguanine (6-TG; ref. 9) which is used in this study. In addition to its established role in repair of postreplicative DNA errors, maintaining genome integrity and cytotoxicity, MMR proteins also have been known to function in the activation of cell cycle checkpoints and the signaling of an apoptotic (type I cell death) response in tumors cells after exposure to several different classes of chemotherapeutic drugs (10, 11).

Recently, another type of cell death, macroautophagy (hereafter called autophagy, type II cell death), has been reported to function as a novel response to some types of DNA damage, such as ionizing radiation (12) and temozolomide (13). However, the molecular mechanisms underlying the autophagic response to DNA damage are still not clearly defined. During autophagy, portions of the cytoplasm are encapsulated in a double-membrane structure called an autophagosome (14). Autophagosomes then fuse with lysosomes in which the contents are delivered, resulting in their degradation by lysosomal hydrolases (15). Under normal physiologic conditions, autophagy occurs at basal levels in most tissues, contributing to the routine turnover of cytoplasmic components (16). It can promote cell adaptation and

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Received 6/22/06; revised 11/13/06; accepted 12/1/06.

Grant support: NIH grants CA050595 and CA112963 (T.J. Kinsella).

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doi:10.1158/1078-0432.CCR-06-1517

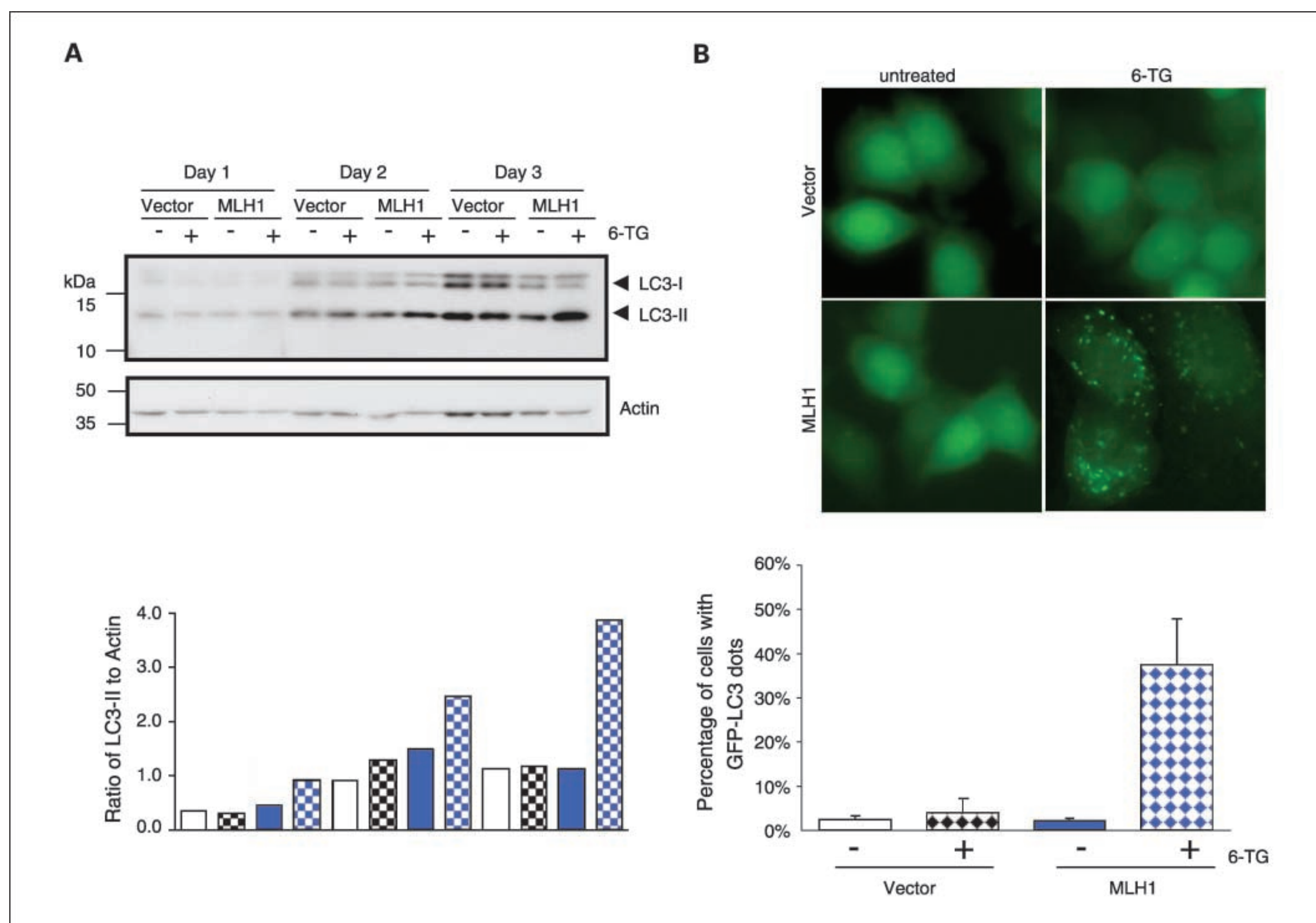

Fig. 1. MLH1 is required for initiating induction of 6-TG – induced autophagy in HCT116 (MLH1+, MMR+) cells. *A,* HCT116 cells stably transfected with an empty vector or with MLH1 cDNA were treated with 3 μmol/L 6-TG for 24 h, harvested, and subjected to immunoblotting analysis with antibodies against LC3 for up to 3 d after 6-TG treatment. *B,* cells were stably transfected with pGFP-LC3 as described in the Materials and Methods. The GFP-LC3 – expressing cells were then treated with 3 μmol/L 6-TG for 24 h and examined under fluorescence microscopy at day 3 after 6-TG treatment. The percentage of GFP-LC3 – positive cells with GFP-LC3 punctate dots was then determined. A minimum of 250 cells per sample were counted from triplicate samples. 6-TG induces a significant increase in autophagic activity in HCT116 (MLH1+, MMR+; $P = 0.0039$, two-tailed t test) but not in HCT116 (vector, MMR−; $P > 0.5$, two-tailed t test). Representative of experiments done at least thrice.

survival during stress, such as starvation; but, under some conditions, cells undergo type II cell death by excessive autophagy (17, 18).

In this report, we investigate the potential role of the MMR system in the autophagic response to 6-TG – induced DNA damage, the function of p53 in the autophagic signaling pathway downstream of MMR, and the effect of autophagy on 6-TG – induced apoptotic cell death. We use two isogenic MMR human tumor cell systems for these studies: MLH1+ versus MLH1− HCT116 cells and MSH2+ versus MSH2− HEC59 cells. HCT116 human colorectal cancer cells are MMR deficient because the *hMLH1* gene in these cells contains a base substitution that results in a termination signal at codon 252 (TCA→TAA; ref. 19). Stable expression of a cloned wild-type *hMLH1* cDNA has been shown to restore MMR activity in the HCT116 cells (20). The human endometrial cancer cell line HEC59, containing mutations in both alleles of *hMSH2*, is also MMR deficient (21). The HEC59/2-4 cell line was established by transfer of a human chromosome 2 containing a wild-type copy of the *hMSH2* gene in the parental cell line and becomes MMR proficient (22). Using these two MMR cell systems, we find that MMR is required for mediating induction of autophagy in response to 6-TG in HCT116 (MLH1+, MMR+) and HEC59 (MSH2+, MMR+) human cancer cells but not in the isogenic MMR− cells. Furthermore, we show that p53 plays an essential role in transferring signals from MMR to the autophagic pathway. Our results also indicate that MMR-initiated autophagy inhibits apoptosis after 6-TG treatment, indicating that the resulting autophagy may function as a mechanism of procell survival.

Materials and Methods

Cells and culture conditions. HCT116 cells stably transfected with an empty vector or human MLH1 cDNA were kindly provided by Dr. Francoise Praz (Centre National de la Recherche Scientifique, Villejuif, France; ref. 20). The human endometrial carcinoma cell lines HEC59 and HEC59/2-4 were kind gifts from Dr. Thomas A. Kunkel (National Institute of Environmental Health, Raleigh, NC; ref. 22). All cells were maintained in DMEM (Mediatech, Inc., Herndon, VA) supplemented with 10% fetal bovine serum (Hyclone, Logan, UT), penicillin (100 μg/mL), streptomycin (100 units/mL), L-glutamine (2 mmol/L), and nonessential amino acids (0.1 mmol/L; Invitrogen, Carlsbad, CA).

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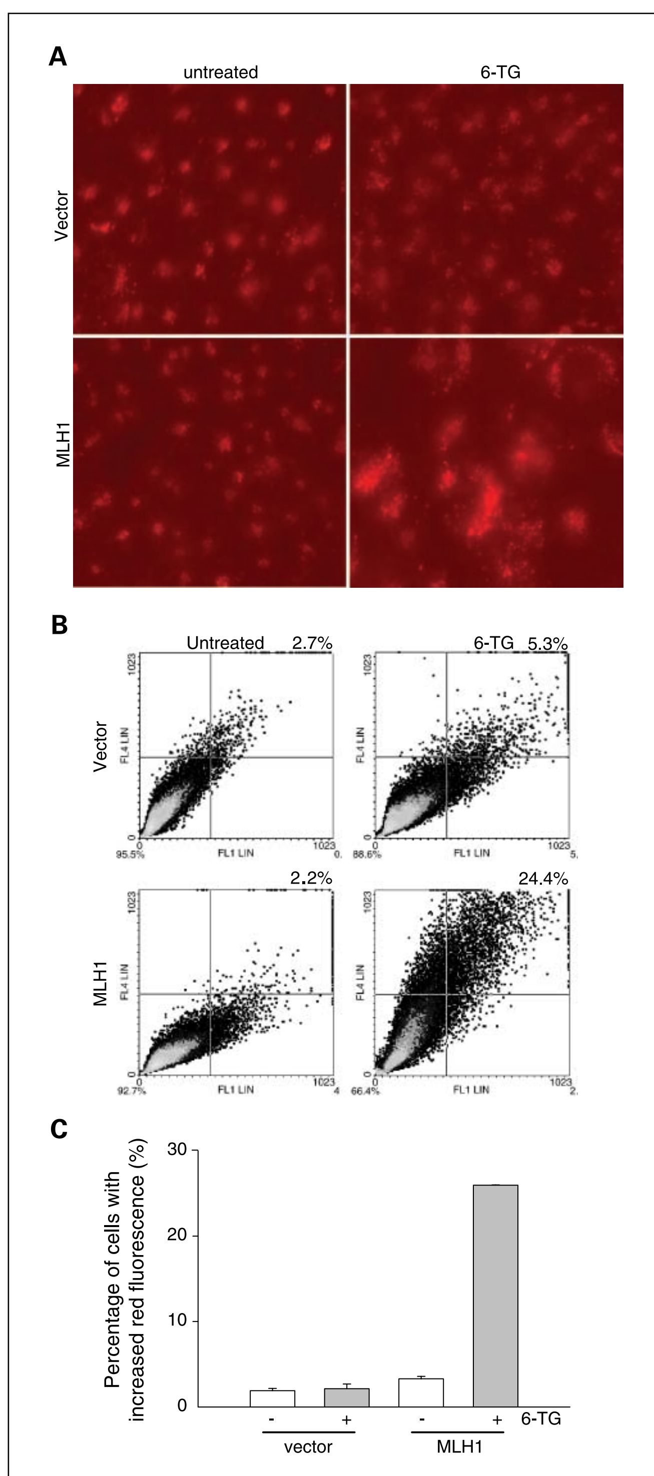

Fig. 2. MLH1 is required for the development of acidic vesicular organelles in HCT116 ($MLH1^+$, MMR^+) cells in response to 6-TG. *A,* HCT116 cells stably transfected with an empty vector or MLH1 cDNA were treated with 3 μmol/L 6-TG for 24 h. Cells were stained with 1 μg/mL acridine orange for 15 min at 3 d after exposure to 6-TG and examined under fluorescence microscopy. *B,* to quantitate the increase in acridine orange red fluorescence, we did a flow cytometric analysis of acridine orange – stained cells using FL4 mode (*Y axis*; >650 nm) to measure bright red fluorescence and FL1 mode (*X axis*; 500-550 nm) to measure green fluorescence. *C,* triplicate samples were done to quantitate the increase in acridine orange red fluorescence as described in (*B*) for statistical analysis. 6-TG induces a significant increase in red fluorescence emitted by acridine orange in HCT116 ($MLH1^+$, MMR^+; $P < 0.001$, two-tailed *t* test) but not in HCT116 (vector, MMR^-; $P > 0.5$, two-tailed *t* test). The experiments were repeated twice.

Drug treatment. Cells were seeded at 30,000 cells/60-mm dish and allowed to attach and grow for ~17 h. 6-TG (3 μmol/L; Sigma, St. Louis, MO) was then added to the medium. After 24 h, 6-TG was removed and the cells were incubated in drug-free medium for the indicated times. To assess the role of p53 in response to 6-TG – induced autophagy, pifithrin-α (BIOMOL, Plymouth Meeting, PA) and 6-TG were added to the medium for 24 h and then pifithrin-α alone was added to the medium for an additional 3 days. Pifithrin-α is known to inhibit p53 transcription activity.

Stable transfection of GFP-LC3 in HCT116 cells. pGFP-LC3 plasmids (a generous gift of Dr. Tamotsu Yoshimori, National Institute of Genetics, Shizuoka-ken, Japan; ref. 23) were transfected into HCT116 (both empty vector and MLH1 cDNA transfected) cells using FuGENE 6 (Roche, Indianapolis, IN), according to the manufacturer's protocol. Forty-eight hours after transfection, the cells were selected with 1 mg/mL G418 for 2 weeks. GFP-LC3 – expressing colonies were isolated and expanded.

Small hairpin RNA – mediated stable silencing of p53 and Atg5 in HCT116 ($MLH1^+$, MMR^+) cells. The pSUPER.retro.puro vector was purchased from OligoEngine (Seattle, WA). The oligonucleotide sequences used for small hairpin RNA interference with p53 and Atg5 expression correspond to nucleotides 775 to 793 (5′-GACTC-CAGTGGTAATCTAC-3′) and to nucleotides 71 to 89 (5′-GGATGAGA-TAACTGAAAGG-3′) downstream of the transcription start site of *p53* (Genbank accession number: AF307851) and *Atg5* (Genbank accession number: NM004849), respectively, followed by a 9-nucleotide non-complementary spacer (TTCAAGAGA) and the reverse complement of the initial 19-nucleotide sequence. A control vector was constructed with a similar insert in which the 19-nucleotide sequence has no homology to any known human gene sequence. The retrovirus was produced in Phoenix-Ampho packaging cells (American Type Culture Collection, Manassas, VA; deposited by Dr. Garry Nolan's laboratory). For transfection, the Phoenix-Ampho cells were seeded at 3×10^6 cells per dish in 60-mm dishes in DMEM containing 10% heat-inactivated fetal bovine serum. After 24 h, the cells were transfected with the pSUPER.retro.puro constructs using LipofectAMINE 2000 reagent (Invitrogen). At 48 and 72 h after transfection, the virus-enriched medium was collected and passed through a 0.22-μm filter. Infections of the MMR^+ HCT116 cells were done on two sequential days in the presence of 4.0 μg/mL hexadimethrine bromide (Sigma). Twenty-four hours after the second infection, the cells were trypsinized and replated in a selection medium containing 1 μg/mL puromycin. After a selection period of 11 days, the puromycin-resistant, infected MMR^+ HCT116 cells were used for studies described in the following sections.

Detection and quantification of acidic vesicular organelles with acridine orange. Vital staining of cells with acridine orange (Molecular Probes, Carlsbad, CA) was done essentially as described (12, 24). Briefly, cells were grown in 60-mm dishes (for fluorescence microscopy) or in 100-mm dishes (for quantification of red fluorescence) and treated with 6-TG or vehicle (NaOH), as described above. Acridine orange was added for 15 min at a final concentration of 1 μg/mL, and unfixed cells were examined immediately by fluorescence microscopy using a Olympus IX71 microscope (Center Valley, PA) with a red filter (excitation 560 nm, emission 645 nm). To quantify red fluorescence emitted by acridine orange, acridine orange stained cells were harvested by trypsinization and immediately analyzed by flow cytometry (12).

Immunoblotting. Cells were lysed in cell lysis buffer, and equal amounts of protein were electrophoresed by standard SDS-PAGE. Proteins were transferred to Immobilon-P membranes (Millipore, Bedford, MA) and the membranes were blocked with 5% nonfat dry milk in TBS containing 0.1% Tween 20. The membranes were then incubated with primary antibody in 5% nonfat dry milk in TBS containing 0.1% Tween 20, followed by incubation with a secondary antibody conjugated to horseradish peroxidase and diluted in 5% nonfat dry milk in TBS containing 0.1% Tween 20. Proteins were detected with SuperSignal West Dura kit (Pierce Biotechnology, Inc., Rockford, IL) and exposed to Fuji films. The primary antibodies used

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for immunoblotting analysis included a rabbit polyclonal antibody against LC3 (a generous gift of Dr. Tamotsu Yoshimori; ref. 23), a rabbit polyclonal antibody against Atg5 (kindly provided by Dr. Noboru Mizushima, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan; ref. 25), mouse monoclonal antibodies against p53 and p21 (Santa Cruz Biotechnology, Santa Cruz, CA), a mouse monoclonal antibody against cleaved poly(ADP-ribose) polymerase (Cell Signaling Technology, Inc., Danvers, MA), a rabbit monoclonal antibody against cleaved caspase 3 (Cell Signaling Technology), and mouse monoclonal antibodies against β-actin and α-tubulin (Sigma).

Statistics. Statistical analysis was done using Student's t test, and $P < 0.05$ was considered significant. Data are expressed as the mean ± SE.

Results

MMR is required for the signaling to the autophagic pathway in HCT116 (MLH1⁺, MMR⁺) cells after exposure to 6-TG. Because MMR is necessary for initiation of the signaling biochemical pathways that lead to apoptosis in response to certain types of chemically induced DNA mispairs, we first determined if MMR is also responsible for mediating induction of autophagy after exposure to 6-TG, a chemotherapy drug recognized by MMR (26). As shown in Fig. 1*A*, HCT116 cells stably transfected with either an empty vector (MMR^-) or with MLH1 cDNA (MMR^+) were treated with 3 μmol/L 6-TG for 24 h. The cells were then harvested and subjected to immunoblotting analysis with antibodies against LC3, a specific marker of mammalian autophagy (23), for up to 3 days after the 6-TG treatment. These data show that human colon cancer MMR^+ HCT116 cells exhibit a progressive increase in autophagic activity (defined by LC3-I to LC3-II conversion) in a time-dependent manner after exposure to 3 μmol/L 6-TG, but the MMR^-HCT116 cells fail to show this autophagic response. To validate the biochemical results from immunoblotting analysis, we also used GFP-LC3 staining to visually detect the cellular changes in autophagic activities. Before induction of autophagy, GFP-LC3 shows diffuse localization in the cytoplasm; whereas when autophagy is induced, GFP-LC3 will exhibit punctate dot cytoplasmic staining (27). Consistent with the results from immunoblotting analysis, ~35% (a significant increase compared with untreated cells, $P = 0.0039$, two-tailed t test) of MMR^+ HCT116 cells show dramatic GFP-LC3 punctate dot staining in response to 6-TG, whereas no significant change in autophagic activity is found in MMR^- HCT116 cells (Fig. 1*B*).

Our results from supravital cell staining with acridine orange are also in agreement with the above data. In acridine orange–stained cells, the cytoplasm and nucleus fluoresce bright green and dim red, respectively, whereas acidic compartments

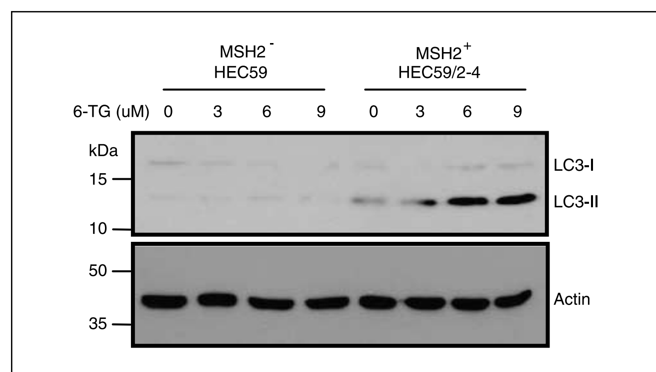

Fig. 3. MSH2 is required for initiating induction of 6-TG–induced autophagy in HEC59 (MSH2⁺, MMR⁺) cells. Human endometrial carcinoma HEC59 (MSH2⁻, MMR⁻) and HEC59/2-4 (MSH2⁺, MMR⁺) cells were incubated with different concentrations (3, 6, and 9 mmol/L) of 6-TG for 24 h. The cells were then harvested and analyzed by immunoblotting with anti-LC3 at day 3 after exposure to 6-TG. The experiment was repeated thrice.

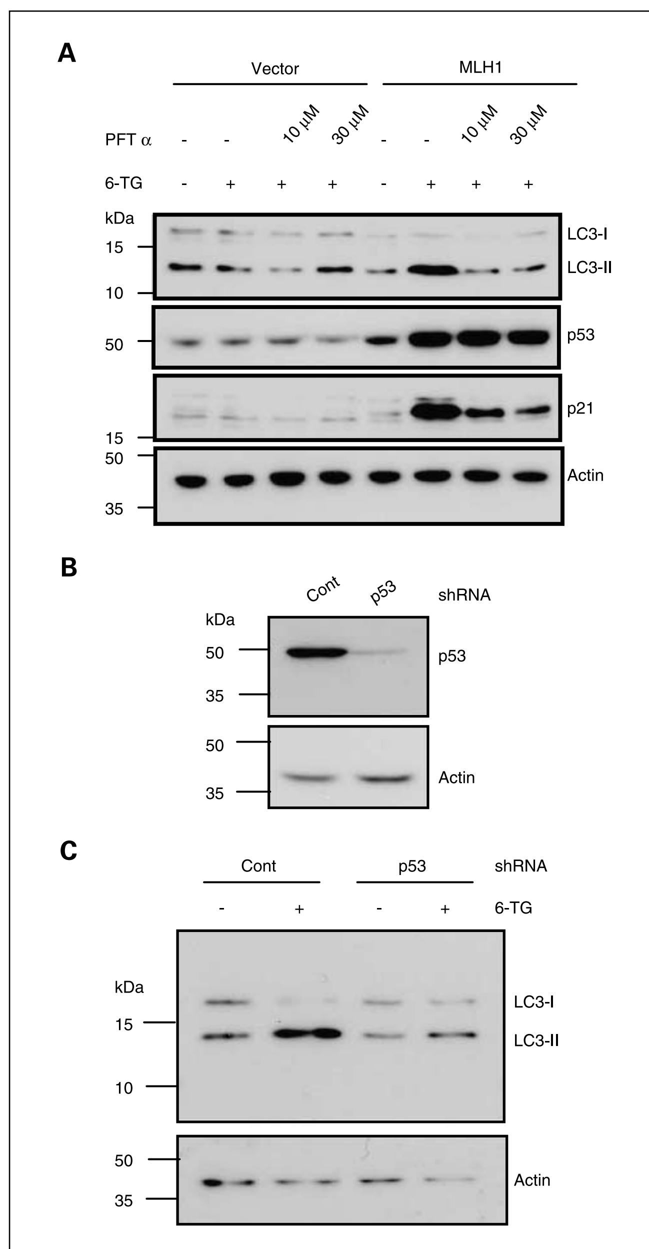

Fig. 4. p53 is essential for transducing signals from MMR to an autophagic pathway in HCT116 (MLH1⁺, MMR⁺) cells after exposure to 6-TG. *A,* HCT116 cells stably transfected with an empty vector or with MLH1 cDNA were treated with 3 μmol/L 6-TG for 24 h. Three days after the treatment, cells were harvested and analyzed by immunoblotting analysis with antibodies against LC3, p53 and p21. To inhibit p53 transcription activity, 10 and 30 μmol/L pifithrin-α were added together with 3 μmol/L 6-TG for 24 h and then alone for another 3 d. *B,* small hairpin RNA–mediated silencing of p53 expression was done as described in the Materials and Methods. p53 expression was tested by immunoblotting analysis with anti-p53 IgG. *C,* control and p53 knockdown HCT116 (MLH1⁺) cells were treated with 3 μmol/L 6-TG for 24 h. Three days after 6-TG exposure, the cells were harvested and subjected to SDS-PAGE and immunoblotting analysis with anti-LC3. The experiments were done thrice.

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fluoresce bright red (28). Autophagy is characterized by the development of acidic vesicular organelles (12, 13, 24). As shown in Fig. 2*A*, vital staining with acridine orange showed the accumulation of acidic vesicular organelles in MMR^+ HCT116 cells at 3 days after a 24-h exposure to 3 μmol/L 6-TG but not in MMR^- HCT116 cells. To quantify the accumulation of acidic vesicular organelles, we did a flow cytometric analysis of acridine orange–stained cells using FL4 mode (>650 nm) to measure bright red fluorescence and FL1 mode (500-550 nm) to measure green fluorescence (12). As shown in Fig. 2*B* and *C*, 6-TG treatment induces a significant increase in the intensity of bright red fluorescence from ~2.2% to ~24.4% in MMR^+ HCT116 cells ($P < 0.001$, two-tailed t test), whereas there is no significant change in MMR^- HCT116 cells ($P > 0.5$, two-tailed t test).

To test if MSH2, another MMR protein, is also required for mediating induction of autophagy in response to 6-TG treatment, the MMR^- human endometrial carcinoma cell line HEC59 ($MSH2^-$) and its isogenic MMR^+ HEC59/2-4 ($MSH2^+$) cells were treated with increasing concentrations of 6-TG (0-9 μmol/L) for 24 h. The cells were then harvested and analyzed by immunoblotting with anti-LC3 on day 3 after the 24-h exposure to 6-TG. These results indicate that 6-TG induces autophagy in a dose-dependent manner in MMR^+ HEC59/2-4 cells but not in MMR^- HEC59 cells (Fig. 3). Taken together (Figs. 1–3), our results suggest that MMR plays an essential role in initiating induction of autophagy in response to 6-TG.

p53 is required for 6-TG–induced autophagy in HCT116 ($MLH1^+$, MMR^+) cells. DNA MMR processing of 6-TG occurs inside the nucleus, whereas autophagy takes place in the cytoplasm. We next addressed the issue of determining how MMR transduces signals from the nucleus to the cytoplasm in 6-TG–induced autophagy. A recent report showed that p53 protein was responsible for mediating the autophagic response to etoposide-induced DNA damage (29). Based on these data, we questioned whether p53 plays a similar role in transferring a MMR-initiated signal to the autophagic pathway. We used pifithrin-α, a p53 inhibitor (30), to determine if p53 functions downstream of MMR in the autophagic signaling pathway. MMR^+ and MMR^- HCT116 cells were treated with 3 μmol/L 6-TG for 24 h. Three days after the treatment, cells were harvested and analyzed by immunoblotting analysis with antibodies against LC3, p53 and p21. To inhibit p53 transcription activity, 0 mmol/L, 10 μmol/L, or 30 μmol/L pifithrin-α were added together with 3 μmol/L 6-TG for 24 h and then pifithrin-α alone was used for another 3 days. As shown in Fig. 4*A*, 10 and 30 μmol/L pifithrin-α, which inhibit p53 transcription activity as measured by inhibition of transactivation of p21 (31), completely abrogates the increase in autophagic activity in MMR^+ HCT116 cells in response to 6-TG. Consistent with the results in Fig. 1, MMR^- HCT116 cells fail to show an increase in autophagic activity after exposure to 6-TG.

To further confirm the role of p53 in 6-TG–induced autophagy, small hairpin RNA–mediated silencing of p53 expression was done in MMR^+ HCT116 cells (Fig. 4*B*). Similar to the above results obtained with the use of a chemical inhibitor of p53, small hairpin RNA–mediated silencing of p53 expression also abrogates 6-TG–induced autophagy in MMR^+ HCT116 cells (Fig. 4*C*). These results suggest that p53 plays an essential role in autophagic response to 6-TG–induced DNA damage in MMR^+ HCT116 cells.

Inhibition of autophagy by Atg5 knockdown promotes apoptosis after MMR processing of 6-TG in HCT116 ($MLH1^+$, MMR^+) cells. The above results show that MMR initiates an autophagic response to 6-TG. We also observed an apoptotic response to

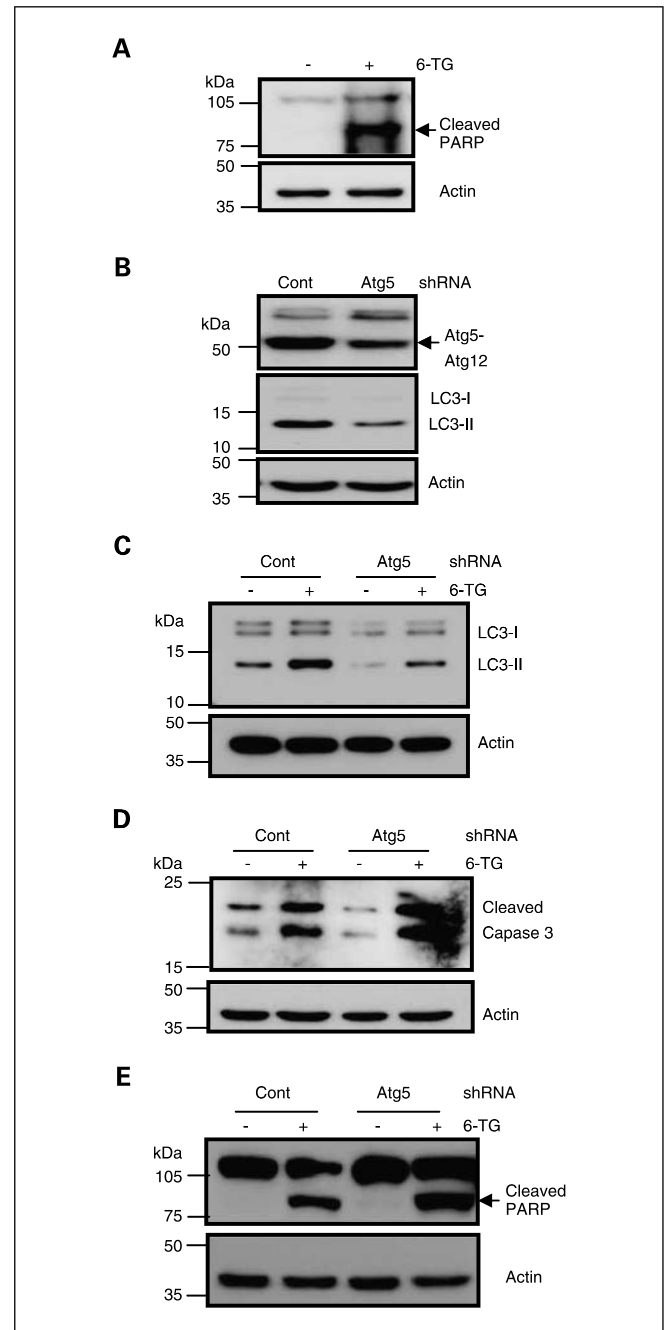

Fig. 5. Inhibition of autophagy by Atg5 knockdown promotes apoptosis in HCT116 ($MLH1^{+,}$ MMR^+) cells after MMR processing of 6-TG. *A*, HCT116 ($MLH1^+$) cells were treated with 3 μmol/L 6-TG for 24 h. Three days after exposure to 6-TG, the cells were harvested and subjected to SDS-PAGE and immunoblotting analysis with anticleaved poly(ADP-ribose) polymerase. *B*, small hairpin RNA – mediated stable silencing of Atg5 expression in HCT116 ($MLH1^+$) cells was done as described in the Materials and Methods. Atg5 and LC3 expression was tested by immunoblotting analysis with anti-Atg5 and anti-LC3 antibodies. Atg5 is conjugated to Atg12 (25). Control and Atg5 knockdown HCT116 ($MLH1^+$) cells were then treated with 3 μmol/L 6-TG for 24 h. Three days after exposure to 6-TG, the cells were harvested and subjected to SDS-PAGE and immunoblotting analysis with anti-LC3 (*C*), anti-cleaved caspase 3 (*D*), and anti-cleaved poly(ADP-ribose) polymerase (*E*). The experiments were repeated twice.

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6-TG in MMR+ HCT116 cells as measured by poly(ADP-ribose) polymerase cleavage, a marker of apoptosis (Fig. 5*A*) under the same condition in which autophagy is induced, which is 3 days after a 24-h 6-TG treatment. This indicates that 6-TG induces both autophagy and apoptosis in MMR+ HCT116 cells. Next, we raised the question whether induction of autophagy affects 6-TG–induced apoptosis. We addressed this question using small hairpin RNA–mediated silencing of Atg5 expression. Atg5 is an essential autophagy gene. It is covalently conjugated to Atg12, and the conjugate is required for the elongation process of isolation membranes and then the formation of autophagosomes (25). Our results show that Atg5 knockdown, which results in a considerable decrease in both basic and 6-TG–induced autophagy (Fig. 5*B* and *C*), dramatically promotes 6-TG–induced caspase 3 and poly(ADP-ribose) polymerase cleavage, markers of apoptosis, in MMR+ HCT116 cells (Fig. 5*D* and *E*). These results suggest that induction of autophagy inhibits apoptosis after MMR processing of 6-TG.

Discussion

MMR proteins play an important role in repairing mismatches caused by DNA polymerase errors. In addition to recognizing and processing DNA mismatches composed of "normal" bases during DNA replication (1, 2), the MMR system also provokes both checkpoint and apoptotic responses after certain types of DNA damage, such as those induced by the Sn1-methylating agents *N*-methyl-*N′*-nitro-*N*-nitrosoguanidine and *N*-methyl-*N*-nitrosourea and by commonly used chemotherapeutic drugs including cisplatin, 5-fluorouracil, temozolomide, and 6-TG (32, 33). In this study, we show that 6-TG induces a dramatic increase in autophagic activity in MMR+ HCT116 and HEC59 cells but not in the isogenic MMR− HCT116 and HEC59 human cancer cells. These results, for the first time, indicate that MMR also initiates an autophagic response besides its known roles in induction of cell cycle arrest and apoptosis.

The tumor suppressor p53 plays a pivotal role in safeguarding the integrity of the genome. p53 is a sequence-specific transcription factor and a central signal integrator of stress, such as DNA damage, hypoxia, and oncogene activation (34). These types of stress activate p53, which in turn initiates cell cycle arrest and programmed cell death (34). After exposure to the methylating agents *N*-methyl-*N′*-nitro-*N*-nitrosoguanidine and *N*-methyl-*N*-nitrosourea, MMR proteins have been shown to bind to O^6-methylguanine adducts, and this binding is thought to trigger the induction of p53 and apoptosis (32). Similar to this response, we found that p53 mediates the signaling to the autophagic pathway after MMR processing of 6-TG–induced DNA damage.

How does MMR initiate the signaling to p53 after MMR processing of 6-TG–induced DNA damage? Our earlier report shows that MMR recognizes and then attempts to excise 6-TG–induced DNA damage, which results in DNA single-strand breaks (26). We speculate that replication protein A, a protein complex, can then bind to ssDNA and recruit the ATR-ATRIP complex to sites of DNA damage (35), thereby mediating the activation of p53 (36). Most of p53 functions are mediated through transcriptional regulation of its target genes (34). Therefore, it will be an important topic for a future study to identify the potential target genes of p53 in the autophagic process after MMR processing of 6-TG.

Several reviews have summarized the recent advances in understanding the role of autophagy in cell survival and cell death (17, 18, 37). Autophagy can promote cell adaptation and survival, but under some conditions, it leads to cell death, depending on the cell type, cellular context, and nature of the treatment (38–40). In our cell system, induction of autophagy inhibits apoptotic cell death after 6-TG–induced DNA damage, probably through the mechanism of degrading damaged mitochondria with loss of membrane potential and, thus, eliminating triggers of apoptosis as suggested in recent reports (41, 42). After 6-TG incorporation into DNA, futile attempt of the MMR system to repair the mismatched base pair results in DNA single-strand breaks, which in turn initiate apoptotic cell death to eliminate the damaged cells (26). At the same time, autophagy might be activated to clear the damaged organelles, such as mitochondria during apoptosis (41, 42), and then inhibit apoptotic cell death. This result suggests that 6-TG–induced autophagy in HCT116 (MLH1+, MMR+) cells may contribute to procell survival after MMR processing of 6-TG–induced DNA mismatches.

In conclusion, we show for the first time that MMR initiates 6-TG–induced autophagy in a p53-dependent manner. The resulting autophagy inhibits apoptosis after MMR processing of 6-TG, indicating that autophagy may have prosurvival effects.

Acknowledgments

We thank Drs. Francoise Praz and Thomas A. Kunkel for providing the cell systems used in this study, Dr. Noboru Mizushima for his generous gift of anti-Atg5, Dr. Tamotsu Yoshimori for providing the antibody against LC3 and pEGFP-LC3 plasmids, all the members of the Kinsella laboratory for their helpful discussions, and the anonymous reviewers for their helpful comments.

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