Apoptotic function of human PMS2 compromised by the nonsynonymous single-nucleotide polymorphic variant R20Q

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Contributed by Richard D. Kolodner, July 2, 2008 (sent for review January 9, 2008)

Mismatch repair (MMR) corrects replication errors during DNA synthesis. The mammalian MMR proteins also activate cell cycle checkpoints and apoptosis in response to persistent DNA damage. MMR-deficient cells are resistant to cisplatin, a DNA cross-linking agent used in chemotherapy, because of impaired activation of apoptotic pathways. It is shown that postmeiotic segregation 2 (PMS2), an MMR protein, is required for cisplatin-induced activation of p73, a member of the p53 family of transcription factors with proapoptotic activity. The human PMS2 is highly polymorphic, with at least 12 known nonsynonymous codon changes identified. We show here that the PMS2(R20Q) variant is defective in activating p73-dependent apoptotic response to cisplatin. When expressed in Pms2-deficient mouse fibroblasts, human PMS2(R20Q) but not PMS2 interfered with the apoptotic response to cisplatin. Correspondingly, PMS2 but not PMS2(R20Q) enhanced the cytotoxic effect of cisplatin measured by clonogenic survival. Because PMS2(R20Q) lacks proapoptotic activity, this polymorphic allele may modulate tumor responses to cisplatin among cancer patients.

chemotherapy | mismatch repair | p73 | cisplatin | MLH1

DNA repair is essential to the maintenance of genome integrity. Defects in DNA repair mechanisms cause developmental abnormalities, premature aging, and cancer (1). Besides their roles in the repair of DNA damage, eukaryotic DNA repair proteins also participate in the transduction of damage signals to regulate cell cycle checkpoints and programmed cell death (2, 3). By virtue of their ability to bind damaged DNA, proteins such as the MRE11–RAD50–NBS1 (MRN) complex and the MSH2–MSH6 (MutS α) complex function as sensors of double-stranded breaks or mismatched base pairs, respectively, to activate repair and checkpoint pathways (4, 5). The dual role of MutS α in repairing mispairs and in activating apoptosis is well established (5). Defect in mismatch repair (MMR) is associated with increased cancer risk, best illustrated with the human cancer syndrome hereditary nonpolyposis colorectal cancer (HNPCC) (6). Among 11 MMR proteins identified to date, defects in MLH1, MSH2, and MSH6 account for 95% of the mutations identified in HNPCC patients (7). Mutations in postmeiotic segregation 2 (PMS2), another MMR gene, have also been linked to increased cancer susceptibility (8).

The core MMR proteins are functionally divided into two groups: the MutS homology (MSH) and the MutL homology (MLH) proteins, named after prokaryotic proteins with similar functions (9). The MutS heterodimers, MutS α and MutS β , consisting of MSH2–MSH6 and MSH2–MSH3, recognize mismatched bases and small insertion/deletion mispairs in DNA (5). The MutS heterodimers initiate DNA repair by recruitment of the MutL α complex, consisting of MLH1 and PMS2, although a second MutL-related complex, MLH1–MLH3, also plays a role in MMR (5). Interaction between MutS and MutL complexes leads to the recruitment of yet other proteins that participate in

MMR (5). Although the biochemistry of prokaryotic MMR is well understood (10), the eukaryotic MMR mechanism is yet to be fully elucidated.

Oxidation, alkylation, or cross-link damage to DNA can activate MMR protein-dependent cell cycle arrest and apoptosis (11–13). The activation of checkpoint and apoptosis by MMR may be the result of either futile repair that generates double-stranded breaks or direct signaling involving interactions between the MMR and other DNA damage response proteins (14). Supporting the direct signaling model are mouse genetic studies showing that the DNA repair and the proapoptotic functions of Msh2 and Msh6 can be separated by specific substitution mutations in each of the two proteins (15, 16). It has also been shown that the checkpoint kinase ATR is recruited to and activated by alkylated DNA through the combined action of MutS α and MutL α , further suggesting a direct role of MMR proteins in DNA damage signal transduction (17).

In a previous report, we showed that the PMS2 subunit of the MutL α complex is required for cisplatin to activate the proapoptotic function of p73 (18). The p73 protein is a transcription factor related to the tumor suppressor p53 and shares the proapoptotic function of p53 (19). Like p53, p73 is activated by DNA damage and can stimulate p53-independent apoptosis (20). The requirement for PMS2 in damage-induced activation of p73 provides yet another piece of evidence for the direct signaling function of MMR proteins. The human PMS2 is particularly polymorphic. A number of single-nucleotide polymorphisms (SNPs) have been reported for the human PMS2, with 19 occurring in the coding sequence, of which 12 SNPs result in nonsynonymous codon changes. We have examined several polymorphic PMS2 variants for their interactions with MLH1 and p73. Here, we show that PMS2(R20Q), a variant encoded by one of the PMS2 polymorphic alleles, is defective in activating p73-dependent apoptosis in response to cisplatin.

Results

Interactions of PMS2 Variants with MLH1 and p73. We examined several PMS2 variants for their interactions with MLH1 and p73

Author contributions: R.D.K., W.E., and J.Y.J.W. designed research; I.M.-T., A.Y.-Y., H.S., E.A., and I.C.H. performed research; H.S. and J.Y.J.W. contributed new reagents/analytic tools; I.M.-T., A.Y.-Y., R.D.K., W.E., and J.Y.J.W. analyzed data; and I.M.-T., R.D.K., and W.E. wrote the paper.

The authors declare no conflict of interest.

Freely available online through the PNAS open access option.

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This article contains supporting information online at www.pnas.org/cgi/content/full/0806435105/DCSupplemental.

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Fig. 1. Polymorphic variants of human PMS2 protein. Shown is a schematic summary of PMS2 protein with five nonsynonymous variants and two mutants (*) examined in this work. NLS, nuclear localization signal; NES, nuclear exporting signal.

(Fig. 1). Previous studies have established that formation of the PMS2–MLH1 heterodimer (MutL α) promotes PMS2 stability (21). Each of the PMS2 variants examined showed increased levels when coexpressed with MLH1 [supporting information (SI) Fig. S1]. PMS2 also interacts with and stabilizes the p73 protein (18). When coexpressed with human TA-p73 α (trans-activating α isoform of p73) (p73), we observed increased levels of p73 with six of the seven variants tested (Fig. 2A and B). However, coexpression of PMS2(R20Q) did not lead to an increase in the p73 protein (Fig. 2A, fourth and fifth lanes, and B, third and fourth bars). These results suggest that PMS2(R20Q) can be stabilized by MLH1, but it does not stabilize p73.

Previous studies have shown that MLH1 and p73 can each stimulate the nuclear accumulation of PMS2 (18, 22). We therefore coexpressed PMS2-yellow fluorescent protein (YFP) and PMS2(R20Q)-YFP fusion proteins with MLH1 or p73 and visualized the subcellular distribution of YFP signals. From 10 randomly chosen fields, averaging 80 cells per field, we counted cells showing exclusively nuclear YFP signal among total YFP-

Fig. 2. Interactions of PMS2 with MLH1 and p73. (A and B) Stabilization of p73 by PMS2. The indicated PMS2 variants were each coexpressed with p73 or appropriate empty plasmid into 293T cells. Whole-cell lysates were used for immunoblotting after β -galactosidase activity normalization of transfection efficiency. (A) Representative immunoblots. (B) Relative levels of p73 in PMS2-cotransfected cells (dark bars) compared with levels in vector-cotransfected cells (white bars). Values shown are from three independent experiments. (C) Nuclear accumulation of PMS2. COS-1 cells were cotransfected with YFP-PMS2 [wild type (wt) or R20Q] with either MLH1 or p73. Cells were stained with Hoechst, and nuclear signal quantification was assessed with fluorescent microscopy. Ten randomly chosen fields were examined, and cells with only nuclear fluorescence signal were counted.

Fig. 3. Effect of cisplatin on PMS2 interaction with MLH1 or p73. (A) Basal interaction of PMS2 and PMS2(R20Q) with p73. Myc-tagged PMS2 (-wt or -R20Q) and HA-tagged p73 α were coexpressed in 293T cells and immunoprecipitated with anti-myc or anti-HA antibodies followed by immunoblotting with anti-myc and anti-HA. (B and C) Cisplatin enhanced interaction of PMS2, but not PMS2(R20Q), with p73. 293T cells cotransfected with HA-p73 and myc-PMS2-wt (B) or myc-PMS2(R20Q) (C) expression plasmids were treated with 0 μ M (lanes 1 and 5), 12.5 μ M (lanes 2 and 6), 25 μ M (lanes 3 and 7), or 50 μ M CDDP (lanes 4 and 8) for 24 h. Cell extracts were immunoprecipitated with anti-myc antibody and then analyzed by anti-myc and anti-HA immunoblotting. (D) Interaction of MLH1 with PMS2 or PMS2(R20Q) is not affected by cisplatin. 293T cells cotransfected with the indicated expression plasmids were treated with 0 μ M (lanes 1 and 4), 25 μ M (lanes 2 and 5), or 50 μ M (lanes 3 and 6) CDDP for 24 h. Cell extracts were immunoprecipitated with anti-myc antibody and analyzed by anti-myc, anti-MLH1, and anti-HA immunoblotting.

positive cells (Fig. 2C). When coexpressed with MLH1, >80% of the YFP-positive cells showed exclusively nuclear YFP signals with either PMS-YFP or PMS2(R20Q)-YFP (Fig. 2C Left). In cells expressing PMS2-YFP or PMS2(R20Q)-YFP alone, none of them showed an exclusively nuclear YFP signal. Coexpression with p73 resulted in nuclear accumulation of PMS2-YFP in $\approx$ 30% of transfected cells (Fig. 2C Right). Coexpression with p73 also induced the nuclear accumulation of PMS2(R20Q)-YFP in $\approx$ 20% of transfected cells. These results suggest that the R20Q substitution does not affect the interaction between PMS2 and MLH1 but reduces the interaction with p73.

Cisplatin Does Not Enhance the Interaction of PMS2(R20Q) and p73.

The interaction between PMS2 and p73 is stimulated in cisplatin-treated cells (18). In cells coexpressing HA-p73 with myc-PMS2 or myc-PMS2(R20Q), we detected a low level of HA-p73 in anti-myc immunoprecipitation (IP) before cisplatin treatment (Fig. 3A). In reciprocal co-IPs, a low level of myc-PMS2 or myc-PMS2(R20Q) was detected in the anti-HA immune complexes (Fig. 3A). These low levels of co-IPs were dependent on the expression of the appropriately tagged proteins, ruling out nonspecific trapping by the anti-myc or the anti-HA antibodies

Fig. 5. PMS2 but not PMS2(R20Q) sensitizes cells to cisplatin. (A) The indicated cell populations were seeded at 200 cells per well of a 6-well dish. The next day they were treated with 8 μ M CDDP for 1 h or 0 μ M CDDP (control). After washing with medium three times, cells were grown for a further 10 days, and colonies were counted after methanol fixation and crystal violet staining. Percentage of colony survival was calculated as the number of colonies in CDDP-treated wells divided by number of colonies in untreated, control wells. The data shown are from four independent experiments, with triplicates per cell type per experiment. (B) Assessing the influence of excess p73 levels on cisplatin-induced cell death. The Pms2-deficient c18.2 cells stably expressing vector, PMS2 (wt), or PMS2(R20Q) were transfected with pYFP-p73 plasmid (bars 1–6) or empty pYFP plasmid (bars 7–12). After 24 h, cells were treated with 0 μ M (control) or 25 μ M CDDP for 24 h, and the uptake of PI among the YFP⁺ cells was measured by FACSCalibur. Data represent four independent experiments with duplicates per cell line per experiment.

percentage of colony survival as the ratio of colony numbers in cisplatin-treated dishes to those in the control dishes (Fig. 5A). The plating efficiency of Pms2-deficient MEFs was between 30 and 35%. After treatment with 8 μ M cisplatin, between 12 and 15% of the Pms2-deficient MEFs survived to form colonies. Expression of PMS2 decreased the clonogenic survival rate to <10% ($P \leq 0.005$), whereas expression of PMS2(R20Q) did not affect the clonogenic survival of MEFs. This result shows that PMS2, but not PMS2(R20Q), sensitizes cells to cisplatin.

PMS2(R20Q)-Defective Response Is Maintained After p73 Overexpression. The Pms2-deficient MEFs contain 5–15 copies of p73 mRNA per cell, as determined by quantitative real-time RT-PCR experiments (data not shown). However, we were unable to detect the mouse p73 protein in these MEFs; as a result, we could not examine the effect of PMS2 or PMS2(R20Q) on the endogenous p73. We therefore tested whether the ectopic expression of p73 could influence the death response to cisplatin in these cells. Pms2-deficient MEFs stably expressing vector, human PMS2 or PMS2(R20Q) were transfected with plasmids expressing YFP-p73 or YFP, treated with or without 25 μ M cis-diamminedichloroplatinum (CDDP) for 24 h, and the uptake of propidium iodide (PI) was measured in the YFP-positive cells by FACS analyses (Fig. 5B). Consistent with previous reports (18, 24), ectopic expression of YFP-p73 increased the levels of PI uptake even without cisplatin treatment (Fig. 5B, compare

CDDP, bars 2, 4, and 6 with 8, 10, and 12). Expression of YFP-p73 also accelerated the death response to cisplatin, such that significant levels of PI-positive cells were detected at 24 h after drug addition (Fig. 5B, compare +CDDP bars 1, 3, and 5 with 7, 9, and 11). In cells expressing PMS2, cisplatin-induced death was significantly higher in the YFP-p73-transfected population (Fig. 5B, compare bars 3 and 9), consistent with previous results (18). By contrast, this cisplatin-induced and YFP-p73-dependent increase in death was not observed with cells expressing PMS2(R20Q) (Fig. 5B, compare bars 1 and 5). These results support the conclusion that PMS2(R20Q) is defective in transducing cisplatin damage signal to p73.

Human PMS2 and PMS2(R20Q) Did Not Correct the MMR Defect of Mouse Pms2-Knockout Cells. It has been reported that the human PMS2 protein cannot correct MMR defects in Pms2-knockout mouse cells (25). We have independently verified that conclusion with the Pms2-knockout cells reconstituted with retroviral vector, human PMS2, or PMS2(R20Q) (Fig. S2). We performed single-cell cloning from the stably infected cell populations and then analyzed two microsatellite markers by using diluted genomic DNA samples. The results showed that cells expressing PMS2 or PMS2(R20Q) displayed microsatellite instability that was comparable with those expressing vector alone (Fig. S2). Thus, the differential apoptotic and clonogenic survival responses to cisplatin occurred in cells that remained defective for MMR.

Discussion

Genetic polymorphism is a contributing factor to the phenotypic diversity of the human population. In the clinical arena, knowing the effects of polymorphic alleles on the cause or the treatment of diseases is of importance, and such knowledge is essential to the development of personalized medicine. The degree of polymorphism among human genes is highly variable and probably related to the gene function and the selective pressures on that function. In this work, we examined several variants of the human PMS2, which is highly polymorphic, and we found one of the variants to exhibit a defect in promoting the cellular apoptotic response to cisplatin. It will be of interest as future work to determine whether the PMS2(R20Q) allele is associated with increased resistance to cisplatin among cancer patients treated with this chemotherapeutic agent.

The PMS2 gene encodes a MutL-homologous protein that forms a heterodimer with MLH1; and the resulting complex, MutL α , interacts with MutS α/β to activate MMR (26, 27). MLH1 deficiency is associated with the development of many cancer types (28, 29), and PMS2 deficiency is associated with HNPCC and Turcot's syndrome (30–32). The tumor suppression function of MutL α is attributed to its essential role in MMR (10). However, MutL α -dependent activation of checkpoint pathways may also contribute to tumor suppression (14). The genetic evidence that DNA repair and proapoptotic functions of MSH2 or MSH6 can be separated by specific substitution mutations have suggested that these two biological functions may involve different conformations of the MutS α complex (15, 16). Our finding that PMS2(R20Q) is proficient in binding MLH1 but deficient in activating p73 is consistent with that idea, i.e., the repair and proapoptotic functions of MutL α may also involve different conformations.

The codon-20 of PMS2 is not conserved among human, mouse, or yeast, in keeping with its being a polymorphic codon in the human PMS2. It is therefore not surprising to find that the R20Q substitution does not affect the PMS2–MLH1 interaction, nor does it affect the basal interaction between PMS2 and p73. Interestingly, however, R20Q disrupts cisplatin-induced PMS2–p73 interaction. According to the crystal structure of the N-terminal 40-kDa fragment of the human PMS2 protein (33), R20 is in the disordered L1 loop that is N-terminal to the highly

conserved ATPase domain found in MutL homologs. By comparison with the crystal structure of the bacterial MutL homodimer (34), the disordered L1 loop is likely to be involved in the PMS2–MLH1 interaction. We propose two possibilities for how this L1 loop may play a role in the cisplatin-enhanced interaction between PMS2 and p73. In the first scenario, the L1 loop may dissociate from MLH1 after recruitment of the MutL α complex to platinum-damaged DNA and thus promotes PMS2 binding to p73. If so, R20Q may hinder this dissociation of PMS2 from MLH1 and thus interfere with the cisplatin-induced interaction between PMS2 and p73. In the second scenario, the L1 loop may contact p73 directly, and R20Q may prevent this contact site from being created in response to cisplatin. Unlike the bacterial MutL protein, the N-terminal PMS2 fragment exhibits ATPase activity as a monomer (33), suggesting that PMS2 may undergo ATP/ADP conformational transitions without MLH1. It is conceivable that the activation of p73 may involve monomeric PMS2 that is dissociated from MLH1 in response to cisplatin. Because monomeric PMS2 is highly unstable (21, 35), formation of the PMS2–p73 complex may require additional factors that remain to be identified.

Materials and Methods

Cell Lines. HEK 293T, Phoenix eco packaging cells (Orbigen), 3T3 derived from *Pms2*^{−/−} MEF (c18.2 line) (36), and COS-1 cells were grown in DMEM supplemented with 10% FBS and penicillin/streptomycin (50 units/ml, each) (Cellgro).

Quantitation of Protein Levels in Transient Cotransfections. 293T cells were cotransfected with the indicated combination of plasmids: one of tested pCMV-myc-PMS2 variants, pCMV-MLH1, pcDNA3-HA-p73, or empty pcDNA3(+) vector (Invitrogen) by using FuGENE 6 (Roche Molecular Biochemicals). Cells were lysed in RIPA buffer, sonicated, and briefly centrifuged at 10,000 × g. To normalize for transfection efficiency, β -galactosidase expression plasmid (pCMV- β -gal) was added to the transfection mixture, and loading of lysates was determined by the level of enzymatic activity in each lysate, to assure loading of equal number of transfected cells. At 48 h after transfection, whole-cell lysates were analyzed by immunoblotting with standard protocol. The relative expression level of proteins was determined by densitometry. Levels of protein expression in vector-cotransfected cells were set as 1.

Nuclear Accumulation Assay. pYFP-C1 (Clontech) was used for the production of YFP-PMS2 or YFP-PMS2 R20Q fusion proteins. COS-1 cells seeded onto sterile coverslips were transfected with YFP-PMS2 or YFP-PMS2(R20Q) and in the same time cotransfected with MLH1, p73, or empty vector for a control. We introduced 0.5 μ g of each plasmid to cells by using FuGENE 6. After 48 h, cells were fixed with 3.7% paraformaldehyde and stained with 1 μ g/ml Hoechst 33258 DNA. Coverslips were washed with PBS and visualized by a DeltaVision deconvolution microscope with Softworks software (Applied Precision). To determine nuclear-only YFP, we used an epifluorescence microscope (Zeiss) at ×400 magnification. Ten randomly chosen fields were counted per experiment.

Cisplatin Treatment of MEFs. c18.2 cells (1×10^6) were seeded in a 6-cm dish, and the culture was incubated overnight, followed by treatment with 25 μ M cisplatin for 24 h. MEFs are mostly arrested in late S and G₂/M at this time point. To induce apoptosis, the cultures were washed with medium and incubated in fresh medium containing 25 μ M cisplatin for another 24 h. Cells were collected

at the indicated time points during the second 24 h of incubation in triplicate for the analyses of cell death.

Clonogenic Survival Assay. Cells were seeded at a density of 200 cells per each well of a 6-well dish in 2 ml of DMEM + 10% FBS. The next day they were treated with 8 μ M CDDP for 1 h or vehicle as control. After washing with medium three times, cells were cultured further for 10 days with fresh medium added at day 5. After 10 days, medium was removed, and cells were washed in PBS and stained for 30 min with staining solution (0.25% crystal violet, 3.5% formaldehyde, 80% methanol). The wells were washed with water, and colonies were counted. The percentage of colony survival was calculated as a number of colonies in CDDP-treated wells divided by a number of colonies in untreated, control wells. Clonogenic survival of *Pms2*-deficient MEFs expressing vector, PMS2, or PMS2(R20Q) was performed in triplicate. Results from four independent experiments are shown.

PI Uptake Assay. c18.2 cells (3×10^5) were seeded in each well of a 6-well dish. Cells were transfected with pYFP-p73 plasmid or empty pYFP plasmid while still in suspension, by using 2 μ g of each plasmid DNA per well in 6 μ l of FuGENE 6. After 24 h, cells were treated with 25 μ M CDDP for 24 h or 0 CDDP (control cells). Cells were harvested (floating and adherent cells), washed with PBS, and stained with 1 μ g/ml PI solution in PBS with 1% FBS. Within 30 min of staining, the uptake of PI in YFP-positive populations was measured by fluorescence-activated cell sorting (FACSCalibur; BD Biosciences). Data represent four independent experiments in duplicate from each experiment.

Flow Cytometry Analysis. At the indicated time points after cisplatin treatment, floating and attached cells were collected, washed with PBS, stained with PI [0.5 μ g/ml PI, 0.1% Triton X-100, 1 mM Tris (pH 8), 0.1 mM EDTA, 0.1% sodium citrate, 50 μ g/ml RNase] for 30 min on ice in the dark and then analyzed by fluorescence-activated cell sorting (FACSCalibur). The percentage of cells with sub-G₁ DNA content was determined by using the CellQuest program. Cells untreated with cisplatin were used as controls for setting the sub-G₁ gate.

Single-Cell Cloning and Microsatellite Instability. *Pms2*-knockout MEFs stably infected with retroviral vector, retroviral-PMS2, or retroviral-PMS2R20Q were plated sparsely and cultured for 3 weeks to allow the growth of individual colonies, which were picked and expanded in culture for a period of 6 weeks, and the expression of PMS2 or PMS2(R20Q) was examined by immunoblotting with anti-PMS2 antibodies. Genomic DNA was prepared by two independently derived clones from each of the three populations. Mutations in microsatellite sequences were assayed by PCR of single target molecules, as is described in *SI Materials and Methods*.

Statistical Analysis. For trypan blue assay and sub-G₁ measurement, statistical significance was determined by repeated-measurement ANOVA and ANOVA for each time point (WinStat). For protein stability assay, a *t* test for independent sample was used. For clonogenic survival and PI exclusion assay, ANOVA was used to determine statistical significance.

Procedures for immunoblotting, co-IP, PMS2 variant construction, recombinant retroviral infection, trypan blue exclusion assay, and microsatellite instability assay are described in *SI Materials and Methods*.

ACKNOWLEDGMENTS. We thank members of the Wang laboratory for support in discussion and technical performances. We thank Dr. M. R. Liskay (Oregon Health Sciences University, Portland, OR) for the gift of *Pms2*-deficient MEF cells. This work was supported by the National Institute for Environmental Health Sciences/National Institutes of Health (to J.Y.J.W. and R.D.K.), the National Cancer Institute/National Institutes of Health (to W.E.), and the Japan Society for the Promotion of Science Research Fellowships for Young Scientists (to A.Y.-Y.).

- Campisi J (2003) Cancer and ageing: Rival demons? *Nat Rev Cancer* 3:339–349.
- Ishikawa K, Ishii H, Saito T (2006) DNA damage-dependent cell cycle checkpoints and genomic stability. *DNA Cell Biol* 25:406–411.
- Wang J-Y, Cho S-K (2004) Coordination of repair, checkpoint, and cell death responses to DNA damage. *Adv Protein Chem* 69:101–135.
- Usui T, Petrini J-H, Morales M (2006) Rad50S alleles of the Mre11 complex: Questions answered and questions raised. *Exp Cell Res* 312:2694–2699.
- Stojic L, Brun R, Jiricny J (2004) Mismatch repair and DNA damage signalling. *DNA Repair* 3:1091–1101.
- Peltomaki P (2001) Deficient DNA mismatch repair: A common etiologic factor for colon cancer. *Hum Mol Genet* 10:735–740.
- Peltomaki P (2005) Lynch syndrome genes. *Fam Cancer* 4:227–232.
- Gryfe R, Gallinger S (2005) Germ-line PMS2 mutations: One hit or two? *Gastroenterology* 128:1506–1509.

- Yang W (2000) Structure and function of mismatch repair proteins. *Mutat Res* 460:245–256.
- Jun S-H, Kim T-G, Ban C (2006) DNA mismatch repair system: Classical and fresh roles. *FEBS J* 273:1609–1619.
- Duckett D-R, et al. (1996) Human MutS α recognizes damaged DNA base pairs containing O⁶-methylguanine, O⁴-methylthymine, or the cisplatin-d (GpG) adduct. *Proc Natl Acad Sci USA* 93:6443–6447.
- Mello J-A, Acharya S, Fishel R, Essigmann J-M (1996) The mismatch repair protein hMSH2 binds selectively to DNA adducts of the anticancer drug cisplatin. *Chem Biol* 3:579–589.
- Ni T-T, Marsischky G-T, Kolodner R-D (1999) MSH2 and MSH6 are required for removal of adenine misincorporated opposite 8-oxo-guanine in *S. cerevisiae*. *Mol Cell* 4:439–444.
- O'Brien V, Brown R (2006) Signalling cell cycle arrest and cell death through the MMR system. *Carcinogenesis* 27:682–692.

15. Yang G, et al. (2004) Dominant effects of an Msh6 missense mutation on DNA repair and cancer susceptibility. *Cancer Cell* 6:139–150.
16. Lin D-P, et al. (2004) An Msh2 point mutation uncouples DNA mismatch repair and apoptosis. *Cancer Res* 64:517–522.
17. Yoshioka K, Yoshioka Y, Hsieh P (2006) ATR kinase activation mediated by MutS α and MutL α in response to cytotoxic O⁶-methylguanine adducts. *Mol Cell* 22:501–510.
18. Shimodaira H, Yoshioka-Yamashita A, Kolodner R-D, Wang J-Y (2003) Interaction of mismatch repair protein PMS2 and the p53-related transcription factor p73 in apoptosis response to cisplatin. *Proc Natl Acad Sci USA* 100:2420–2425.
19. Ozaki T, Nakagawara A (2005) p73, a sophisticated p53 family member in the cancer world. *Cancer Sci* 96:729–737.
20. Levrero M, et al. (2000) The p53/p63/p73 family of transcription factors: Overlapping and distinct functions *J Cell Sci* 113:1661–1670.
21. Chang D-K, et al. (2000) Steady-state regulation of the human DNA mismatch repair system. *J Biol Chem* 275:18424–18431.
22. Wu X, Platt J-L, Cascalho M (2003) Dimerization of MLH1 and PMS2 limits nuclear localization of MutL α . *Mol Cell Biol* 23:3320–3328.
23. Kaina B (2003) DNA damage-triggered apoptosis: Critical role of DNA repair, double-strand breaks, cell proliferation, and signaling. *Biochem Pharmacol* 66:1547–1554.
24. Gong J-G, et al. (1999) The tyrosine kinase c-Abl regulates p73 in apoptotic response to cisplatin-induced DNA damage. *Nature* 399:806–809.
25. Gibson S-L, et al. (2006) Overexpression of the DNA mismatch repair factor, PMS2, confers hypermutability and DNA damage tolerance. *Cancer Lett* 244:195–202.
26. Nicolaides N-C, et al. (1995) Genomic organization of the human PMS2 gene family. *Genomics* 30:195–206.
27. Kolodner R-D, Marsischky G-T (1999) Eukaryotic DNA mismatch repair. *Curr Opin Genet Dev* 9:89–96.
28. Ricciardone M-D, et al. (1999) Human MLH1 deficiency predisposes to hematological malignancy and neurofibromatosis type 1. *Cancer Res* 59:290–293.
29. Wang Q, et al. (1999) Neurofibromatosis and early onset of cancers in hMLH1-deficient children. *Cancer Res* 59:294–297.
30. De Rosa M, et al. (2000) Evidence for a recessive inheritance of Turcot's syndrome caused by compound heterozygous mutations within the PMS2 gene. *Oncogene* 19:1719–1723.
31. Agostini M, et al. (2005) Two PMS2 mutations in a Turcot syndrome family with small bowel cancers *Am J Gastroenterol* 100:1886–1891.
32. Hamilton S-R, et al. (1995) The molecular basis of Turcot's syndrome *N Engl J Med* 332:839–847.
33. Guarne A, Junop M-S, Yang W (2001) Structure and function of the N-terminal 40-kDa fragment of human PMS2: A monomeric GHL ATPase. *EMBO J* 20:5521–5531.
34. Ban C, Yang W (1998) Crystal structure and ATPase activity of MutL: Implications for DNA repair and mutagenesis. *Cell* 95:541–552.
35. Yao X, et al. (1999) Different mutator phenotypes in Mlh- versus Pms2-deficient mice. *Proc Natl Acad Sci USA* 96:6850–6855.
36. Baker S-M, et al. (1995) Male mice defective in the DNA mismatch repair gene PMS2 exhibit abnormal chromosome synapsis in meiosis. *Cell* 82:309–319.