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Differential Radiosensitization in DNA Mismatch Repair-Proficient and -Deficient Human Colon Cancer Xenografts with 5-Iodo-2-pyrimidinone-2'-deoxyribose

Yuji Seo, Tao Yan, Jane E. Schupp, Valdir Colussi, Kerri L. Taylor, and Timothy J. Kinsella
Department of Radiation Oncology, Case Comprehensive Cancer Center/University Hospitals of Cleveland and Case Western Reserve University, Cleveland, Ohio

ABSTRACT

Purpose: 5-Iodo-2-pyrimidinone-2'-deoxyribose (IPdR) is a pyrimidinone nucleoside prodrug of 5-iododeoxyuridine (IUdR) under investigation as an orally administered radiosensitizer. We previously reported that the mismatch repair (MMR) proteins (both hMSH2 and hMLH1) impact on the extent (percentage) of IUdR-DNA incorporation and subsequent in vitro IUdR-mediated radiosensitization in human tumor cell lines. In this study, we used oral IPdR to assess in vivo radiosensitization in MMR-proficient (MMR⁺) and -deficient (MMR⁻) human colon cancer xenografts.

Experimental Design: We tested whether oral IPdR treatment (1 g/kg/d for 14 days) can result in differential IUdR incorporation and subsequent radiosensitization after a short course (every day for 4 days) of fractionated radiation therapy, by using athymic nude mice with an isogenic pair of human colon cancer xenografts, HCT116 (MMR⁻, hMLH1⁻) and HCT116/3-6 (MMR⁺, hMLH1⁻). A tumor regrowth assay was used to assess radiosensitization. Systemic toxicity was assessed by daily body weights and by percentage of IUdR-DNA incorporation in normal bone marrow and intestine.

Results: After a 14-day once-daily IPdR treatment by gastric gavage, significantly higher IUdR-DNA incorporation was found in HCT116 (MMR⁻) tumor xenografts compared with HCT116/3-6 (MMR⁺) tumor xenografts. Using a tumor regrowth assay after the 14-day drug treatment and a 4-day radiation therapy course (days 11–14 of IPdR), we found substantial radiosensitization in both HCT116 and HCT116/3-6 tumor xenografts. However, the sensitizer enhancement ratio (SER) was substantially higher in HCT116 (MMR⁻) tumor xenografts (1.48 at 2 Gy per fraction, 1.41 at 4 Gy per fraction), compared with HCT116/3-6 (MMR⁺) tumor xenografts (1.21 at 2 Gy per fraction, 1.20 at 4 Gy per fraction). No substantial systemic toxicity was found in the treatment groups.

Conclusions: These results suggest that IPdR-mediated radiosensitization can be an effective in vivo approach to treat “drug-resistant” MMR-deficient tumors as well as MMR-proficient tumors.

INTRODUCTION

5-Iodo-2-pyrimidinone-2'-deoxyribose (IPdR) is a pyrimidinone nucleoside and an oral prodrug of 5-iododeoxyuridine (IUdR) under preclinical, and soon to be clinical, investigation by our group (1–5). Orally administered IPdR is converted to the active drug, IUdR, by an aldehyde oxidase localized mainly in the liver (6). IUdR is a halogenated thymidine analogue and can be incorporated into DNA, replacing thymidine. IUdR has been recognized as an effective in vitro in vivo radiosensitizer since the 1960s (7–14); however, its clinical use is limited by a narrow therapeutic window when the drug is given as a continuous infusion (2). We have previously shown that oral IPdR treatment can improve the therapeutic index in comparison with continuous infusion IUdR by using an athymic nude mice model with several different human tumor xenografts (1).

Mismatch repair (MMR) deficiency is associated with hereditary nonpolyposis colon cancer and an increasing number of sporadic cancers (15). In addition, the MMR system has been linked to regulating the DNA damage response to various types of chemotherapy (16, 17). A substantial amount of preclinical and clinical data has established that the MMR-deficiency in human tumors is associated with resistance (tolerance) to alkylating agents (18, 19), 6-thioguanine (20), 5-fluoropyrimidines (21, 22), doxorubicin (23), and cis-platinum (24, 25). Therefore, there is a compelling need to develop novel therapeutic strategies for MMR-deficient (MMR⁻) tumors.

In contrast to the clear evidence of chemotherapeutic drug resistance, it remains controversial whether the MMR-mediated damage response pathway alters ionizing radiation (IR)-induced cell death. Our in vitro data with high-dose-rate IR indicate that the MMR influences an IR-induced cell cycle response (i.e., MMR-proficiency results in a more profound and sustained G2-M arrest; ref. 26), but this response was not associated with a significant difference in clonogenic survival in vitro (27). Other investigators report that MMR-deficiency results in decreased survival after high-dose-rate IR linking MMR to the
homologous recombination repair pathway (28, 29). Alternatively, increased survival was found in isogenic MMR-deficient (MMR−) murine cell lines after low-dose-rate IR, compared with MMR-proficient (MMR+) cells (30, 31). The molecular mechanisms behind these different cellular responses to MMR processing of IR damage are not completely understood.

We have been interested in the potential use of IPdR/IUdR-mediated radiosensitization for MMR-deficient cancers. We previously demonstrated that MMR proteins (both hMSH2 and hMLH1) impact on the extent (percentage) of IUdR-DNA incorporation and subsequent IUdR-mediated radiosensitization in vitro (32–35). The mechanism of IUdR-mediated radiosensitization is an augmentation of IR-induced DNA damage, and the extent of radiosensitization is generally considered to correlate with the percentage of IUdR-DNA incorporation (36–39). Comparing various pairs of isogenic MMR− and MMR+ human cancer and murine cell lines, MMR− cell lines consistently showed a significantly higher percentage of IUdR-DNA incorporation and greater radiosensitizing effects by high-dose-rate IR after IUdR exposure (32–35). The main purpose of this study was to extend our observations to an animal model with an isogenic pair of human tumor xenografts differing in MMR status and to test whether oral IPdR treatment results in differential drug incorporation in DNA and subsequent radiosensitization with a short course (every day for 4 days) of fractionated radiation therapy (RT), comparing the response of MMR− versus MMR+ tumors in the same animal.

**MATERIALS AND METHODS**

**Cell Lines and Tumor Xenografts.** An isogenic pair of human colorectal cancer cell lines [HCT116: MMR-deficient (hMLH1−); HCT116/3-6: MMR-proficient (hMLH1+)] were maintained in DMEM supplemented with 10% fetal bovine serum, L-glutamine, and penicillin/streptomycin at 37°C in a humidified 10% CO2 atmosphere. We have previously characterized the enzymatic activities for thymidine kinase, thymidylate synthase, and aldehyde oxidase, as well as the deoxynucleotide triphosphate pools in these isogenic human tumor cells, and have found similar levels (32). In a preliminary study, the *in vitro* cell doubling times of HCT116 and HCT116/3-6 were measured to be 23.5 ± 0.8 and 21.4 ± 3.6 hours (mean ± SD), respectively. In the presence of IUdR (3 μmol/L) in the medium, the doubling time of HCT116 and HCT116/3-6 increased to 27.3 ± 1.5 and 29.1 ± 2.7 hours, respectively. Cells were passed twice weekly to maintain exponential growth. For *in vitro* IUdR-DNA incorporation and cell cycle analysis, the exponentially growing cells were seeded at 1 × 106 cells per 100-mm plate, and IUdR exposure was begun as indicated.

For tumor xenografts, the cell lines were harvested with trypsinization. Three × 106 cells were injected into subcutaneous tissue in the right (HCT116/3-6) and left (HCT116) lower flanks of athymic nude mice (6–8 weeks of age). In preliminary experiments, we measured the *in vivo* tumor volume doubling times and found them to be similar (HCT116: 6.1 ± 1.1 days; HCT116/3-6: 6.0 ± 0.8 days). The growth of the tumor xenografts was monitored daily by measuring perpendicular tumor diameters. Ten days after subcutaneous injection of the isogenic pair of tumor cells, drug administration was begun.

**Drug Preparation and Dose/Schedule.** IPdR was synthesized and obtained from SuperGen Inc. (Pleasanton, CA). IPdR was suspended daily in 10% gum arabic in PBS and administered to the mice by gastric gavage. The dose of IPdR was 1 g/kg/d given once daily for 14 consecutive days. A control group of mice was given the same volume of vehicle once daily by gastric gavage for 14 consecutive days. In a separate experiment, mini-osmotic pumps (Alzet model 2001, DURECT Co., Cupertino, CA) for continuous infusion of IUdR were implanted in the subcutaneous tissue of the dorsal flank of the mice with tumor xenografts. IUdR (Sigma, St. Louis, MO) was dissolved in 0.1 mol/L NaOH, and were stored at −80°C. IUdR was delivered continuously at the dose of 100 mg/kg/d for 7 days, which is the maximum tolerated dose, as we reported previously (3).

**IUdR-DNA Incorporation Assay.** Groups of mice with tumor xenografts were given IPdR for 14 days followed by a 14-day drug-free period. During the total 28-day experimental period, mice were sacrificed on days 3, 7, 14, 17, and 28. The tissues (tumor xenografts, small intestine, femurs) were harvested and stored at −80°C until analysis. The tissues of mice receiving continuous infusion IUdR (100 mg/kg/d for 7 days) were sacrificed on day 7, and the same tissues were harvested. After IPdR or IUdR experiments, the percentage of IUdR-DNA incorporation was determined according to the method of Blander et al. (40) with minor modifications as described previously (1). At the time of analysis, the tissues were thawed and minced in PBS. The tissues were homogenized with a sonic dismembrator followed by DNA isolation and enzymatic hydrolysis. A solution containing nucleosides and the analogue was analyzed by high performance liquid chromatography (HPLC) with a reversed-phase column. HPLC analysis was done with a Waters System controller 600E, Autosampler 717, Multispectrum detector 400E, and a 3.9 × 300-mm μBondapak C18 reverse phase column (Waters Co., Milford, MA). The mobile phase consisted of 20 mmol/L sodium acetate (pH 2.0) with 8% acetonitrile at a flow rate of 1.0 mL/min. UV absorption at 260 nm and 290 nm detected thymidine and IUdR peaks, respectively, and quantification was performed against authentic nucleoside standards with Millennium32 software v. 3.05.01 (Waters Co.). The typical retention times of thymidine and IUdR were 6 and 9 minutes, respectively. The percentage of IUdR-DNA incorporation was calculated by the formula:

\[
\frac{\text{IUdR}}{[\text{IUdR}] + [\text{Thymidine}]} \times 100
\]

**Plasma Drug Concentration.** IPdR (1g/kg) was administered to separate groups of athymic nude mice by gastric gavage to determine plasma pharmacokinetics after a single dose. Approximately 40 μL of blood was drawn and collected in Microvette tubes CB300 (Sarstedt Inc., Newton, NC) by a saphenous vein puncture at 0.25, 0.5, 1, 2, 4, and 8 hours after IPdR administration by gastric gavage. The plasma was separated by centrifugation at 5000 × g for 10 minutes. The nucleoside analogues were extracted by the method described previously (1) and were analyzed by HPLC. The HPLC instruments used were the same as in the IUdR-DNA incorporation assay described above. The mobile phase consisted of 20 mmol/L...
sodium acetate (pH 4.0) with a 2-to-8% linear gradient of acetonitrile at a flow rate of 1.0 mL/min. The peaks of IUdR and IPdR were detected by UV absorption at 290 nm and 335 nm, respectively.

In vitro Cell Cycle Analysis. Exponentially growing HCT116 and HCT116/3-6 cells were exposed to IUdR (3 or 30 μmol/L) for 24 or 48 hours (≈1–2 cell doubling times). The cells were then harvested by trypsinization and fixed with cold 70% EtOH. For propidium iodide staining, the cells were incubated in 33 μg/mL propidium iodide, 1 mg/mL RNase, 0.5 mmol/L EDTA, and 0.2% NP40 overnight. Flow cytometry analysis was done with a Coulter EPICS XL-MCL (Coulter Co., Miami, FL). At least 20,000 events were analyzed with ModFit LT 2.0 (Verity, Inc., Topsham, ME).

Fractionated Radiation Therapy and Tumor Regrowth Assay. Groups of mice with both HCT116 and HCT116/3-6 xenografts were treated with oral IPdR every day for 14 days by gastric gavage. On days 11 to 14 of IPdR administration, RT was delivered to the tumor xenografts with 2 Gy or 4 Gy per fraction for 4 days. For irradiation, the mice were immobilized without anesthesia and 6-MV photon beams were delivered to the tumors with a linear accelerator (Mevatron MD, Siemens AG, New York, NY) with 1.5-cm bolus. The adjacent normal tissues were shielded by a half beam lead block. Each tumor xenograft was treated each day with separate fields. After irradiation, the tumor size (the maximum and its rectangular diameter) and the body weight were monitored daily until the tumors grew to ≦300% of the

Fig. 1  A, the percentage IUdR-DNA incorporation in vitro. The cells were treated with 3 μmol/L IUdR for 24 or 48 hours. The percentage of IUdR-DNA incorporation in HCT116 was significantly higher than that in HCT116/3-6 at both 24 and 48 hours (P = 0.05; t test, two-tail). The experiments were repeated twice (error bars, SEM). In B, athymic nude mice were treated with a 7-day continuous subcutaneous infusion of IUdR at 100 mg/kg/d (n = 14; error bars, SEM). C, kinetics of the percentage IUdR-DNA incorporation in the tumor xenografts (HCT116 and HCT116/3-6), small intestine, and bone marrow during the 14-day IPdR oral (p.o.) treatment and for a 14-day drug elimination period (n = 10–12 at each time point; error bars, SEM). D, in vivo comparison of the percentage of IUdR-DNA incorporation in HCT116 (MMR -) with that in HCT116/3-6 (MMR +). Connected by lines, pairs of tumor xenografts in the same mice. A significantly higher incorporation was found in HCT116 (MMR +) on day 14 (*) (P < 0.05, two-tail paired t test).
RESULTS

Drug Incorporation In vitro and In vivo. In previous studies (32, 33), we showed differential IUdR-DNA incorporation with various isogenic pairs of MMR− and MMR+ cell lines. Fig. 1A shows the percentage of IUdR-DNA incorporation in HCT116 (MMR−, hMLH1+) and HCT116/3-6 (MMR−, hMLH1+) cells treated in vitro with 3 μmol/L IUdR for 24 or 48 hours (1–2 doubling times). There was a statistically significant difference between the two cell lines at 24 and 48 hours (P = 0.05; t test, two-tail). HCT116 cells show an approximately 50% increase in IUdR-DNA incorporation compared with HCT116/3-6 cells, consistent with our previous results (32, 33). We next tested the time course of IUdR-DNA incorporation in HCT116 (MMR−) tumor xenografts versus HCT116/3-6 (MMR+) tumor xenografts and normal proliferating tissues (bone marrow, intestine) with IPdR orally administered for 14 days (Fig. 1C) as well as IUdR continuous subcutaneous infusion for 7 days with the previously established maximum tolerated dose (ref. 41; Fig. 1B). Because both tumor xenografts were implanted in each animal, the data on the percentage of IUdR-DNA incorporation in the tumor xenografts after IPdR treatment were also compared by a paired t test (Fig. 1D). Throughout both experiments with continuous infusion IUdR or daily IPdR, the percentage of IUdR-DNA incorporation tended to be higher in HCT116 (MMR−) xenografts than in HCT116/3-6 (MMR+) xenografts. The difference between the two tumor xenografts reached a substantial level on day 14 of the IPdR treatment (1–2 doubling times). In the IPdR drug-free period (days 17, 21, 28; Fig. 1D), the differences were not substantial, although the trend to higher incorporation in the MMR-deficient tumor xenografts remained.

During the IPdR treatment, the percentage of IUdR-DNA incorporation in the small intestine was at a level similar to that in the tumors. The percentage of IUdR-DNA incorporation in bone marrow was approximately one half of that in the tumors (Fig. 1C). This is in contrast to the use of continuous infusion IUdR, which leads to a 2- to 4-fold higher percentage of IUdR-DNA incorporation in these two normal proliferating tissues compared with the tumor xenografts (Fig. 1B). These in vivo data regarding the lower percentage of IUdR-DNA incorporation in bone marrow and small intestine after IPdR treatment (Fig. 1C) are of clinical relevance because myelosuppression and diarrhea are the two dose-limiting normal tissue toxicities of continuous infusion IUdR, as determined in prior clinical trials (8, 10–14). Interestingly, the percentage of IUdR-DNA incorporation in small intestine decreased more rapidly during the IPdR drug elimination period (days 14–28) in comparison with the tumor xenografts, demonstrating a potentially enhanced therapeutic ratio for tumor radiosensitization after drug administration (Fig. 1C). A similar time course effect of normal intestinal drug elimination (“wash-out”) was found in a previously reported clinical trial with continuous infusion 5-bromodeoxyuridine (42).

Plasma Drug Concentration. Plasma IPdR and IUdR concentrations after a single dose of IPdR with 1 g/kg are shown in Fig. 2. The peak concentration of IUdR was 63.1 ± 14.8 μmol/L at 15 minutes. Plasma IUdR level declined rapidly to 6.0 μmol/L at 2 hours and 0.7 μmol/L at 8 hours after IPdR administration. For comparison, the maximum tolerated dose (100 mg/kg/d) of continuous infusion IUdR × 7 days in athymic mice resulted in a steady-state plasma IUdR concentration of 1.5 ± 0.2 μmol/L, as previously reported (1, 3, 41). Thus, the use of oral IPdR results in dramatically higher but transient plasma IUdR levels in comparison with continuous infusion IUdR.

Cell Cycle Analysis In vitro. There are accumulating data that connect MMR and a G2-M arrest in response to genotoxic agents (21, 43, 44). Although IUdR incorporation into DNA is a type of base damage, IUdR is generally less cytotoxic in comparison with other base analogues like 6-thioguanine (32–34), and a short-term pulse exposure (4-hour) of IUdR did not change cell cycle progression in either MMR− cells or MMR+ cells, as we previously reported (33). In this study, we further examined whether continuous exposure of IUdR to 3- and 30-μmol/L concentrations for 24 or 48 hours, (1–2 cell doubling times) might result in alterations of the cell cycle in an MMR-dependent manner (Fig. 3). In both HCT116 and HCT116/3-6 cells, there was a similar decreased in the S-phase fraction in a dose- and time-dependent manner. G1 arrest was evident in HCT116 (MMR−), whereas G2-M arrest was more pronounced in HCT116/3-6 (MMR+). However, these differential cell cycle alterations were subtle, and the biological significance seems unclear at the lower IUdR concentration (3 μmol/L). The higher IUdR concentration of 30 μmol/L was cytotoxic for both cell lines as determined by cell viability assays (data not shown).

Fig. 2 Plasma pharmacokinetics of IUdR and IPdR after a single oral administration of IPdR with 1 g/kg. The data represent the average of 5 athymic nude mice (±SEM).
concentration (30 μmol/L) did show more pronounced cell cycle effects. These in vitro data raise a question regarding possible in vivo cell cycle effects on both tumor and normal tissues during the 14-day IPdR treatment schedule, in which IUdR plasma concentrations would be predicted to be ≥30 μmol/L (Fig. 2) for at least 2 hours each day. We elaborate on this question in the Discussion section.

Fractionated Radiation Therapy and Tumor Regrowth Assay. Fig. 4 shows the tumor regrowth data after fractionated RT (either 2 Gy or 4 Gy per day for 4 days) with or without the 14-day IPdR treatment (Fig. 4A, HCT116; Fig. 4B, HCT116/3-6). Tumor regrowth delay was measured by the area between the 300% volume lines of the specific treatment groups compared with that for its controls. Table 1 shows the averages (95% confidence intervals) of the areas calculated from the data of individual tumors (10–18 in each group). A larger value indicates a greater delay of tumor regrowth.

Comparing the tumor regrowth data after IPdR + RT with 2 Gy or 4 Gy every day for 4 days, with RT alone, substantial increases in regrowth delay were found in both HCT116 and HCT116/3-6 tumor xenografts. To quantitate the extent of radiosensitization by IPdR, the sensitizer enhancement ratio (SER) was calculated as the ratio of the area of [IPdR + RT] versus the area of [RT]. Because IUdR incorporation into DNA results in a more pronounced G2-M delay in HCT116/3-6 than in HCT116 in vitro (Fig. 3), the value was normalized by the ratio of the area of [IPdR alone] versus the area of [control] to adjust the potential direct effect of IPdR drug alone on the regrowth delay. Namely:

$$SER = \frac{\text{Area}_{\text{IPdR + RT}}}{\text{Area}_{\text{RT}}} \times \frac{\text{Area}_{\text{control}}}{\text{Area}_{\text{IPdR alone}}}$$

On the basis of these assumptions and calculations, HCT116 (MMR−) tumor xenografts showed substantially greater SERs than did HCT116/3-6 (MMR+) tumor xenografts (Table 2).
To further examine the differential response to fractionated RT with or without IPdR, the tumor regrowth delay was assessed in each individual mouse (carrying both MMR+/H11001 and MMR-/H11002 tumors) with a paired Student’s t-test. This analysis also revealed that HCT116 (MMR-/H11002) xenografts were substantially more sensitive than HCT116/3-6 (MMR+/H11001) xenografts to IPdR/H11001 RT (Table 1). However, because there was a tendency of greater tumor growth delay in the MMR+/H11001 tumor with IPdR alone, the MMR system seemed to have less impact on the total effects for tumor regrowth (including IR, direct drug effect, and radiosensitization) than on the true radiosensitizing effect, i.e., SERs. Additionally, we found that RT alone with 4 Gy per day for 4 days demonstrated a substantially higher tumor regrowth delay in HCT116 (MMR-) xenografts than did HCT116/3-6 (MMR+) xenografts (Fig. 4; Table 1).

**Systemic Toxicity.** Systemic toxicity was assessed by a measurement of body weight each day during the 2 weeks of drug treatment (and/or 4 days of RT) and for 2 weeks after treatment (Fig. 5). Both RT-alone and IPdR-alone treatment groups showed mild to moderate body weight loss (~10% body weight change). No differences in body weight changes were noted with either 2 Gy per fraction or 4 Gy per fraction for 4 days, and both RT groups were combined in this analysis of systemic toxicity. The combined (IPdR + RT) treatment group experienced similar levels of weight loss, and no additional adverse effects were found. The weight loss was transient in all of the treatment groups, returning to control weights within 5 to 7 days.
7 days after treatment. There was no treatment-related mortality. For comparison, we previously demonstrated that continuous infusion IUDr at 100 mg/kg/d for 7 days results in up to 20% weight loss during treatment (3, 41).

DISCUSSION

In this study, we found favorable differential radiosensitization with a 14-day IPdR treatment schedule in HCT116 (MMR−) tumor xenografts compared with HCT116/3-6 (MMR+) tumor xenografts (Fig. 4; Tables 1 and 2). No significant systemic toxicity was noted during, and for 2 weeks after, IPdR treatment (Fig. 5), similar to our preclinical toxicology study of IPdR in ferrets (5). The improved therapeutic gain for orally administered IPdR compared with continuous infusion IUDr results from lower percentage of IUDr-DNA incorporation in bone marrow and intestine as well as higher percentage of IUDr-DNA incorporation in human tumor xenografts (Fig. 1; refs. 1–5).

We found that greater radiosensitization in the MMR− tumor xenografts correlates with higher levels of IUDr-DNA incorporation in the MMR+ tumors compared with the MMR tumors. This result is consistent with our prior in vitro data (32–35). We have shown recently that the MMR system can recognize IUDr-DNA incorporation as IUDr-A and/or IUDr-G DNA mispairs (35). We speculate that MMR actively removes (repairs) these IUDr-DNA mismatches, supporting our in vitro data that MMR− tumor cells retain higher levels of IUDr-DNA incorporation, which leads to greater radiosensitization in MMR− tumors compared with MMR+ tumors (Fig. 4; Tables 1 and 2). We also found previously that the processing of IUDr-DNA incorporation (damage) by MMR appears independent of p53 status (34) but may involve cooperation with base excision repair (BER; ref. 45). We reported that XRCC1 plays a role in determining the extent of IUDr-DNA incorporation and radiosensitization (45). We are currently investigating the role of MED1, another BER enzyme, in IUDr-DNA processing, based on a recent report showing that the MED1 protein interacts with MLH1 protein in response to genotoxic stress (46). These in vitro data suggest that a combination of IPdR and a chemical inhibitor of BER such as methoxamine could lead to even greater in vivo radiosensitization in MMR− human tumors. This combination is currently being tested in our laboratory.

Another possible mechanism of the observed differential radiosensitization in MMR− versus MMR+ tumor xenografts may be that MMR mediates IR damage responses independent of IUDr-DNA incorporation. As mentioned in the Introduction, there is considerable debate in the literature regarding the role of MMR in an IR-induced DNA damage response. IR can cause various types of DNA damage, and MMR-mediated cellular responses after IR damage seem to vary, depending on IR dose, dose rate, and type of cell model used (26–31). Our previous in vitro data suggest that the MMR system, although affecting the extent of a G2-M arrest, has no significant impact on clonogenic survival after high-dose-rate IR (27). Other investigators found small but significant differences, when the MMR− cells show higher survival after high-dose-rate IR (28). It was also reported that MMR deficiency increased clonogenic survival with low-dose-rate IR (30, 31). In contrast, it has been recently shown that the MMR-proficiency leads to higher survival after high-dose-rate IR, especially at higher IR doses, in association with the DNA double-strand break repair pathway (29). Our in vivo data, indeed, demonstrate a modest but significantly greater radiosensitivity in the MMR-deficient tumor xenografts compared with the MMR-proficient tumor xenografts with 4-Gy-for-4-days RT, whereas no significant difference was found with 2 Gy for 4 days (Fig. 5; Table 1). Further experimental studies are needed to better assess a differential effect (if any) on MMR processing of IR damage in vivo.

We also questioned whether drug effects on the cell cycle related to the transient high IUDr levels (≥30 µmol/L for 2 hours; Fig. 2) after oral IPdR administration might alter the radiation response of the tumor xenografts. This question is

### Table 1  Tumor regrowth assay

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>IPdR alone</th>
<th>RT2Gy</th>
<th>IPdR+RT2Gy</th>
<th>RT4Gy</th>
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<td>2020</td>
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<td>6193</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>*P &lt; 0.01</td>
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<tr>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>*P &lt; 0.05</td>
<td></td>
<td>*P &lt; 0.05</td>
</tr>
</tbody>
</table>

Note. The values represent the mean (95% confidence intervals) of the area between the 300% tumor volume lines of each treatment group compared with its control.

* P < 0.05; ** P < 0.05; *** P < 0.05 (paired t test, two-tail).

### Table 2  The SER calculated from the data of the tumor regrowth assay for HCT116 (MMR−) and HCT116/3-6 (MMR+) human tumor xenografts

<table>
<thead>
<tr>
<th>RT dose</th>
<th>HCT116</th>
<th>HCT116/3-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 Gy × 4</td>
<td>1.48 (1.45–1.51)</td>
<td>1.41 (1.38–1.44)</td>
</tr>
<tr>
<td>4 Gy × 4</td>
<td>1.21 (1.18–1.24)</td>
<td>1.20 (1.17–1.24)</td>
</tr>
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* Average (95% confidence intervals).
based on a large experimental database that connects the MMR system to a G2-M arrest after DNA damage induced by various types of chemotherapeutic drugs including N-methyl-N'/H11032-nitro-N'-nitrosoguanidine (16, 17), 6-thioguanine (43), fluoropyrimidines (21), and cisplatin (44). Previously, we examined the in vitro cell cycle progression in these same MMR/H11001 cells versus MMR/H11002 human tumor cells with a pulse exposure of IUdR (1–10 μmol/L for 4 hours), which revealed no cell cycle alterations in either cell line, although there was a significant difference in the amount of IUdR-DNA incorporation (32). In this study, we used a longer IUdR exposure for one to two cell population doublings (i.e., 24 or 48 hours) to 3 or 30 μmol/L IUdR (Fig. 3). The lower concentration of IUdR (3 μmol/L), which is a clinically relevant steady-state plasma level as a continuous exposure of IUdR (8), showed only a small alteration in the G2-M population. However, the higher concentration (30 μmol/L) showed a more significant differential G2-M cell cycle arrest in MMR/H11001 cells (Fig. 3). This observation may support the hypothesis that the MMR system is involved in DNA damage response induced by IUdR-DNA incorporation. However, the question still remains as to whether the oral IPdR treatment schedule could lead to differential in vivo cell cycle alterations in the G2-M phase, which might contribute to the differential radiosensitivity in MMR− tumor xenografts compared with MMR+ cells (Fig. 4, Table 1). Although plasma pharmacokinetic analysis showed orally administered IPdR (1 g/kg) resulted in the peak plasma concentration of more than 50 μmol/L, the plasma IUdR level declined rapidly over the next 6 to 8 hours after drug administration. On the basis of the pharmacological data (Fig. 2; refs. 1, 2, 4, 5), we conclude that a differential in vivo cell cycle response in the G2-M phase with the IPdR treatment would probably not have a substantial effect on radiosensitization.

In conclusion, a 14-day schedule of IPdR at 1g/kg/d resulted in substantial radiosensitization in both the MMR-deficient and -proficient tumor xenografts. However, the extent of radiosensitization was consistently higher in the MMR-deficient tumor xenografts compared with MMR-proficient tumor xenografts. Given the reduced systemic toxicity of oral IPdR compared with continuous infusion IUdR compared with continuous infusion IUdR (1–5), we conclude that the IPdR-mediated radiosensitization can be an effective approach to treat “drug-resistant” MMR-deficient tumors as well as MMR-proficient tumors.

REFERENCES


Differential Radiosensitization in DNA Mismatch Repair-Proficient and -Deficient Human Colon Cancer Xenografts with 5-Iodo-2-pyrimidinone-2′-deoxyribose

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