

ATM-Deficient Colorectal Cancer Cells Are Sensitive to the PARP Inhibitor Olaparib¹



Chen Wang*, Nicholas Jette*, Daniel Moussienko*, D. Gwyn Bebb[†] and Susan P. Lees-Miller^{*,†}

*Department of Biochemistry & Molecular Biology, Robson DNA Science Centre, Arnie Charbonneau Cancer Institute, University of Calgary, Calgary, Alberta, Canada;

[†]Department on Oncology, Tom Baker Cancer Centre, Calgary, Alberta, Canada

Abstract

The ataxia telangiectasia mutated (ATM) protein kinase plays a central role in the cellular response to DNA damage. Loss or inactivation of both copies of the ATM gene (*ATM*) leads to ataxia telangiectasia, a devastating childhood condition characterized by neurodegeneration, immune deficiencies, and cancer predisposition. *ATM* is also absent in approximately 40% of mantle cell lymphomas (MCLs), and we previously showed that MCL cell lines with loss of ATM are sensitive to poly-ADP ribose polymerase (PARP) inhibitors. Next-generation sequencing of patient tumors has revealed that *ATM* is altered in many human cancers including colorectal, lung, prostate, and breast. Here, we show that the colorectal cancer cell line SK-CO-1 lacks detectable ATM protein expression and is sensitive to the PARP inhibitor olaparib. Similarly, HCT116 colorectal cancer cells with shRNA depletion of ATM are sensitive to olaparib, and depletion of p53 enhances this sensitivity. Moreover, HCT116 cells are sensitive to olaparib in combination with the ATM inhibitor KU55933, and sensitivity is enhanced by deletion of p53. Together our studies suggest that PARP inhibitors may have potential for treating colorectal cancer with ATM dysfunction and/or colorectal cancer with mutation of p53 when combined with an ATM kinase inhibitor.

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Introduction

A driving principle of precision medicine is to exploit genetic differences in tumor tissue to eradicate cancer cells while sparing the normal tissue. A prime example of this concept is the use of poly-ADP ribose polymerase (PARP) inhibitors to target tumors with alterations in the breast and ovarian cancer susceptibility genes *BRCA1* and *BRCA2*. This concept, termed synthetic lethality, after the genetic principle of the same name [1], arose from the demonstration that *BRCA1*- or *BRCA2*-deficient breast cancer cell lines are sensitive to small molecule PARP inhibitors [2,3]. Subsequently, clinical trials showed that the PARP inhibitor olaparib is effective in breast, ovarian, and prostate cancers with *BRCA* deficiency, and olaparib has been approved for use in patients with *BRCA*-deficient ovarian cancer [4–6].

BRCA1 and *BRCA2* are required for repair of DNA double strand breaks (DSBs) via the homologous recombination (HR) pathway (reviewed in [7]). Accordingly, siRNA depletion of other proteins involved in HR as well as other DSB repair pathways, including the protein kinase ataxia-telangiectasia mutated (*ATM*), renders cell lines

sensitive to PARP inhibition [8]. Because the *ATM* gene is altered in many human cancers (Figure 1 and Table 1 [9,10]), we hypothesized that PARP inhibitors might also have efficacy in cell lines with tumor-derived loss or mutation of *ATM*. Up to 40% of mantle cell lymphoma (MCL) cases have loss of *ATM* [11], and we showed that MCL cell lines with low *ATM* protein expression are sensitive to olaparib in both cell line and animal models [12]. Similar results were

Address all correspondence to: Susan P. Lees-Miller, Department of Biochemistry & Molecular Biology, Robson DNA Science Centre, Arnie Charbonneau Cancer Institute, University of Calgary, Calgary, Alberta, Canada.

E-mail: leesmill@ucalgary.ca

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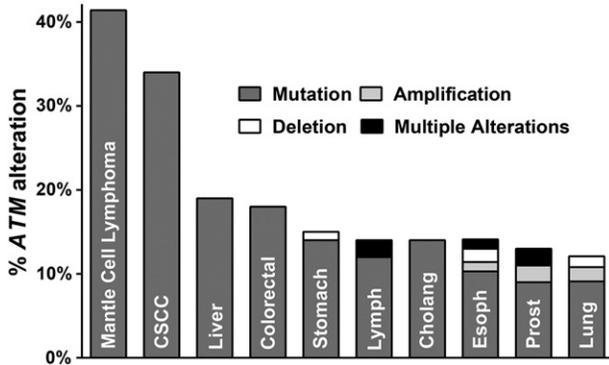


Figure 1. Predicted rates of ATM and p53 alteration in CRC. Redrawn from c-Bioportal [20,21], accessed November 2016. From left to right: MCL, cutaneous squamous cell carcinoma, liver hepatocellular carcinoma, colorectal adenocarcinoma, stomach adenocarcinoma, lymphoid neoplasm diffuse large B-cell lymphoma, cholangiocarcinoma, esophageal carcinoma, prostate adenocarcinoma, and lung adenocarcinoma.

observed in chronic lymphocytic leukemia cells [13]. Moreover, sensitivity to olaparib in ATM-deficient MCL cell lines was enhanced when p53 was also deleted or inactivated [14]. ATM is also mutated in ~12.5% of gastric cancers [15,16], and we found that low ATM protein expression correlates with olaparib sensitivity in gastric cancer cell lines [17]. Moreover, depletion of ATM by shRNA rendered p53-deficient gastric cancer cells highly sensitive to olaparib *in vitro* [17]. Low ATM expression was also shown to correlate with better outcome in a clinical trial of gastric cancer patients treated with olaparib and paclitaxel [18]. Thus, PARP inhibitors could be beneficial in the treatment of cancers not only with BRCA deficiency but also with defects in ATM and possibly other DSB repair genes. Indeed, in a phase 2 clinical trial, four out of five men with metastatic prostate cancer whose tumors carried mutations in ATM responded to the PARP inhibitor olaparib [6].

Colorectal cancer (CRC) is the third most common cancer in men and women with an estimated 134,490 new cases of CRC diagnosed in the United States in 2016 [19]. Analysis of genome sequencing data using c-Bioportal [20,21] revealed that ATM is mutated in approximately 10% of CRCs (Figure 1A). Recognizing that gene alterations do not necessarily correspond to loss of function, these

Table 1. Predicted Rates of ATM Alteration in New Cancer Cases in the United States in 2016

Cancer Site	Estimated New Cancer Cases 2016	Average % Cancer with ATM Alteration	Estimated # Patients with ATM Alteration
Breast	249,260	3.07	7652
Bladder	76,960	7.82	6018
Colorectal*	134,490	10	13,449
Kidney	62,700	3.15	1975
Lung	224,390	7.35	16,492
Melanoma	76,380	7.75	5919
Myeloma	30,330	2.4	728
Ovarian	22,280	4.45	991
Pancreas	53,070	4.76	2526
Prostate	180,890	8.79	15,900
Thyroid	64,300	3.7	2379

Estimated new cancer cases in the United States in 2016 were taken from [19]. Reported rates of ATM alteration in various cancer types were averaged from values in c-Bioportal [20,21], accessed November 2016.

* Colon and rectal combined.

observations prompted us to ask whether ATM deficiency might sensitize CRC cells to PARP inhibitors. Given the reported range of ATM alteration and CRC incidence, we estimate that, in 2016, over 13,000 newly diagnosed CRC patients in the United States could carry alterations in ATM and therefore might benefit from treatment with a PARP inhibitor (Table 1). In addition, it is estimated that almost 80% of CRCs carry alterations in TP53 [22,23], leading us to speculate that p53-deficient CRCs might also be targeted by using a PARP inhibitor in combination with an ATM inhibitor, as we have shown previously in gastric cancer and MCL cell lines [14,17]. Thirty to fifty percent of CRCs also carry activating mutations in K-Ras [24], but the effects of Ras activation on susceptibility of ATM-deficient cells to PARP inhibitors have not been addressed.

Here we screened a panel of CRC cell lines for ATM expression and function. Our results show that the CRC cell line SK-CO-1 has low ATM protein expression and is sensitive to olaparib. Moreover, HCT116 cells with shRNA depletion of ATM are sensitive to olaparib, and deletion of p53 enhances this sensitivity. In keeping with our previous studies [14,17], the combination of olaparib plus the ATM inhibitor KU55933 [25] enhanced sensitivity in CRC cells, particularly when TP53 was deleted. Together, our findings suggest that CRC patients with mutations in ATM may benefit from introduction of PARP inhibitors and that CRC patients with inactivating mutations in p53 could benefit from treatment with PARP inhibitors in combination with ATM inhibitors.

Results

In our previous studies, we have shown that MCL and gastric cancer cell lines with depletion or inactivation of ATM are sensitive to PARP inhibitors, particularly when p53 was also mutated or deleted [14,17]. To test this hypothesis in CRC, we first analyzed ATM and p53 protein expression in the CRC cell lines HCT116, SNU-C1, LS123, SK-CO-1, T84, and LoVo. HCT116 cells carry a mutation (c3380C>T) in one ATM allele, whereas SK-CO-1 cells are homozygous for c.2251-10T>G (Table 2), but the effects of these mutations on ATM protein expression and function have not been determined. HCT116, LoVo, and SK-CO-1 are wild type for TP53, whereas LS123 and SNU-C1 are homozygous for mutant TP53 and T84 is heterozygous for mutant TP53. All cell lines except SNU-C1 contained at least one copy of mutant K-Ras (Table 2). Extracts from a lymphoblastoid cell line of an ataxia telangiectasia (A-T) patient (L3) or their age-matched control (BT) were used as negative and positive controls, respectively, for ATM expression [26]. ATM protein expression was highest in LoVo and T84 cells followed by LS123 and SNU-C1 cells, followed by HCT116 and SK-CO-1 cells (Figure 2). Expression in HCT116 cells was approximately half of that in T84, LS123 and LoVo cells, suggesting that the mutant allele might affect ATM expression, whereas expression in SK-CO-1 cells was virtually undetectable, similar to that in cells from an A-T patient (L3) (Figure 2, A and B). p53 protein was detected in HCT116, SK-CO-1, LoVo, and SK-CO-1 cells but not in SNU-C1 or T84 cells (Figure 2).

To assess ATM functionality in the DNA damage response, cells were irradiated with 2 Gy IR; harvested after 1 hour; and analyzed for ATM serine 1981 autophosphorylation [27], p53 serine 15 phosphorylation [28], and other markers of the DNA damage response by Western blot. IR-enhanced ATM S1981 phosphorylation was observed in BT (matched control for L3 cells from normal, ATM-proficient individual), HCT116, SNU-C1, LS123, T84, and

Table 2. Characterization of CRC Cell Lines Used in This Study

Cell Line	<i>ATM</i> [†]	ATM protein [*]	ATM p1981 [*]	<i>TP53</i> [†]	TP53 protein	<i>KRAS</i> [†]
HCT116	W/M (c.3380C>T sub/mis)	+	+	W/W	+	W/M (c.38G>A sub/mis)
LoVo	W/W	+	+	W/W	+	W/M (c.38G>A sub/mis)
LS123	W/W	+	+	M/M (c.524G>A sub/mis)	++	W/M (c.34G>A sub/mis)
SK-CO-1	M/M (c.2251-10T>G sub/int)	-	-	W/W	+	M/M (c.35>T sub/mis)
SNU-C1	W/W	+	+	M/M (c.497C>A sub/mis)	-	W/W
T84	W/W	+	+	W/M (c.1136G>C sub./mis)	-	W/M (c.38G>A sub/mis)

W, wild type. M, mutant. Values in parenthesis indicate mutations, where known.

Sub = base substitution. Mis = missense mutation. Int = intronic.

-, no protein detected by immunoblot (this study). +, protein expression detected by immunoblot (this study). ++, high protein expression detected by immunoblot (this study).

^{*} This study.

[†] From COSMIC database.

LoVo cells but not in L3 or SK-CO-1 cells, consistent with functional ATM in SNU-C1, LS123, T84, and LoVo cells but not in SK-CO-1. ATM-1981 phosphorylation was observed in HCT116 cells, albeit at a low level, suggesting that ATM is functional in these cells despite low levels of protein expression and one mutant allele (Table 2). IR-induced p53 serine 15 phosphorylation was observed in HCT116 and LoVo cells consistent with expression of wt-p53 and functional ATM, whereas high levels of constitutively phosphorylated p53 were observed in LS123 cells consistent with mutant p53 and functional

ATM (Figure 3). No p53 serine 15 phosphorylation was detected in SK-CO-1 cells consistent with lack of functional ATM (Figure 3).

Having demonstrated that SK-CO-1 cells lack functional ATM, we next asked whether SK-CO-1 cells were sensitive to olaparib. For these experiments, we first compared SK-CO-1 cells (no detectable ATM activity) to LoVo cells (capable of DNA damage-induced autophosphorylation of ATM). Both these cell lines have wild-type p53 (Table 2), but p53 serine 15 phosphorylation was not detected in SK-CO-1 cells, consistent with loss of ATM function. SK-CO-1 cells were more sensitive to olaparib than LoVo cells at all doses tested (Figure 4). We previously reported that MCL cells with loss of ATM but wt-p53 were sensitive to olaparib and that depletion of p53 enhanced olaparib sensitivity [14]. In contrast, shRNA depletion of ATM in gastric cancer cells had little effect on olaparib sensitivity unless p53 was also deleted [17]. The finding that SK-CO-1 cells are sensitive to olaparib despite the presence of wt-p53 (Figure 4) suggests that wt-p53 is less of a barrier to PARP sensitivity in ATM-deficient CRC cells than in ATM-deficient gastric cancer cells.

Because LoVo and SK-CO-1 cells differ in K-Ras status (Table 2) and, as cancer cell lines, likely have additional mutations, it was important to further test our hypothesis in an isogenic background in which p53 was either present or absent. To address this question, we chose to deplete ATM from HCT116 cells either expressing wt-p53 (HCT116 p53^{+/+}) or in which p53 has been deleted (HCT116 p53^{-/-}) [29]. As noted above, HCT116 cells contain one mutant copy of *ATM*, but they express ATM protein that undergoes IR-induced autophosphorylation at site serine 1981 [27] suggesting that they retain ATM activity (Table 2, Figure 3). HCT116 p53^{+/+} and HCT116-p53^{-/-} cells were transfected with shRNA to ATM or control shRNA to GFP, and stable cell lines were isolated as described previously [14,17]. Absence of p53 and/or ATM protein was confirmed by Western blot in the respective cell lines (Figure 5A). We then determined the sensitivity of ATM-depleted HCT116 cells either with wild-type p53 or lacking p53 to olaparib as described above. HCT116 parental and control cells expressing shGFP were relatively resistant to olaparib, showing over 20% cell survival at 3 μM olaparib, whereas depletion of ATM reduced survival to approximately 0.3% at 3 μM despite the presence or absence of p53 (Figure 5, B and C). However, at lower concentrations of olaparib, the surviving fraction in shATM p53^{+/+} cells was higher than in shATM p53^{-/-} cells, for example, compare 1% survival at 2 μM olaparib in HCT116 shRNA ATM p53^{+/+} cells to 0.2% survival in HCT116 shRNA ATM p53^{-/-} cells (Figure 5, B and C). These results confirm that depletion of ATM results in olaparib sensitivity in CRC cell lines and that sensitivity is enhanced when p53 is also deleted.

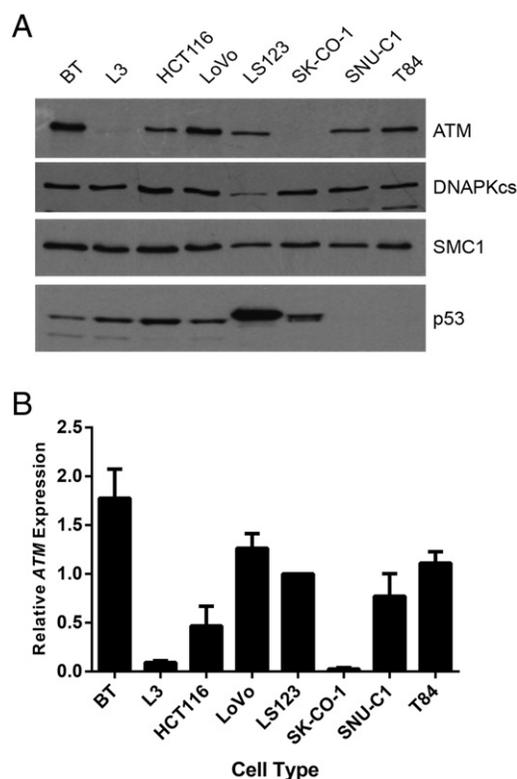


Figure 2. The colorectal cell line SK-CO-1 lacks ATM protein expression. (A) Extracts from human CRC cell lines were generated by NETN lysis, and 50-μg aliquots of total protein were analyzed by SDS-PAGE and immunoblotting to antibodies as shown on the right-hand side. Results are representative of three separate experiments. (B) Quantitation of ATM protein levels in the CRC cell lines, normalized to SMC1, from three separate experiments. ATM protein expression (normalized to SMC1) in LS123 cells was set as one.

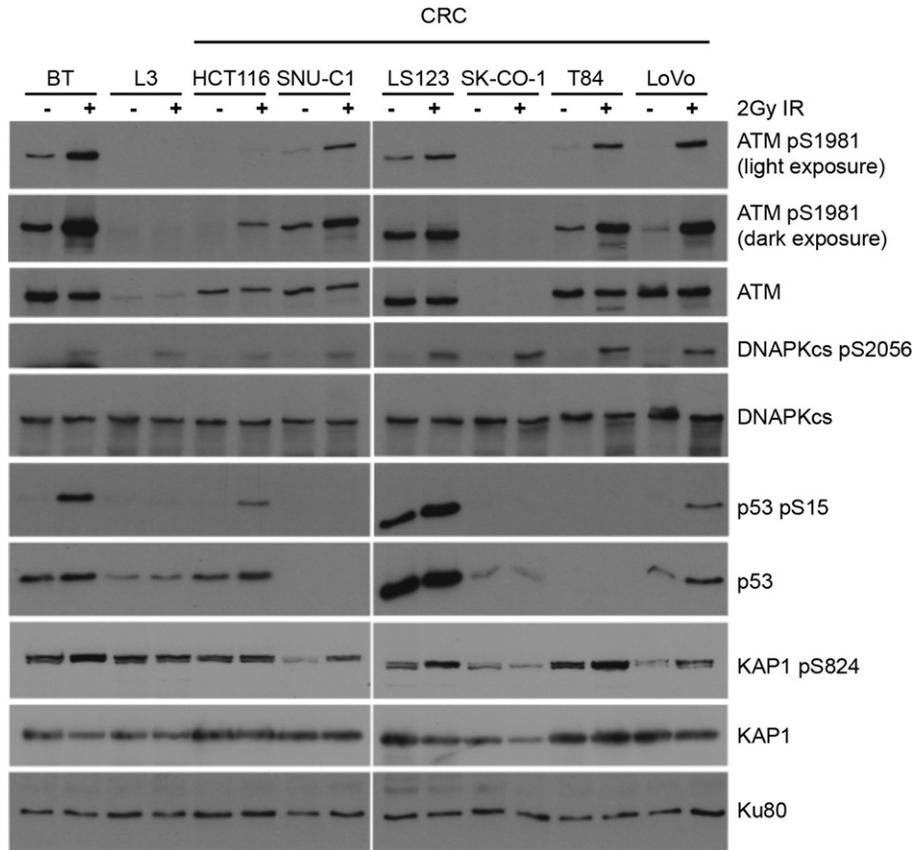


Figure 3. Analyses of DNA damage response proteins in colorectal cell lines by immunoblotting. Human CRC cell lines (HCT116, SNU-C1, LS123, SK-CO-1, T84, and LoVo) and ATM-proficient and ATM-deficient human lymphoblastoid cells (BT and L3, respectively) were treated with 2 Gy IR and harvested 1 hour later. Whole cell lysates were prepared and analyzed by immunoblotting as in Figure 2. The figure shows results from one experiment run under identical conditions on two separate gels (indicated by the white line). For each gel, exposures were for the same time under the same conditions.

Because the majority of CRC cells express wild-type *ATM* but have mutation of *TP53* [22,23], we also examined whether the *ATM* inhibitor KU55933 [25] sensitized *ATM*-proficient but *p53*-deficient CRC cells to olaparib. HCT116 cells with either wt-*p53* or deletion of *p53* were incubated with 0, 1, 2, or 3 μM olaparib in the absence or presence of 2.5, 5, or 7.5 μM KU55933, and survival was determined by clonogenic survival assays. In HCT116 cells with wt *p53*, addition of 7.5 μM KU55933 resulted in approximately 5% survival at 3 μM olaparib, whereas in HCT116 *p53*^{-/-} cells, survival was reduced to approximately 0.2% (Figure 6). Thus, inhibition of *ATM* protein kinase activity enhances olaparib sensitivity in CRC cell lines, particularly when *p53* is deleted.

Discussion

ATM is a serine/threonine protein kinase that plays a major role in regulating the cellular response to DNA DSBs [30,31]. In addition, roles for *ATM* in regulation of oxidative stress, metabolic syndrome, neurodegeneration, and other cellular processes have been reported (reviewed in [32]). Germline mutations in *ATM* lead to ataxia telangiectasia (A-T), a devastating childhood condition characterized by loss of neuromuscular control, immune defects, cancer predisposition, and premature aging [33]. However, next-generation sequencing of tumor samples has revealed that *ATM* is frequently mutated in many sporadic cancers, providing a potential opportunity to exploit genetic differences between normal and tumor tissue to enhance killing of *ATM*-deficient tumor cells [34,35]. In addition, as

a protein kinase with a central role in the DNA damage response, *ATM* is an attractive therapeutic target, and consequently, small molecular inhibitors of *ATM* kinase activity have been developed [25,36,37].

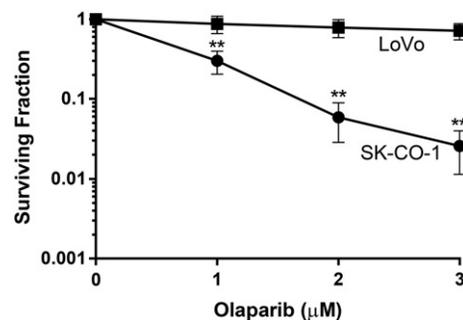


Figure 4. The *ATM*-deficient SK-CO-1 CRC cell line is sensitive to olaparib. Cells were counted, seeded onto 6-cm dishes, and allowed to adhere overnight. The PARP inhibitor olaparib or DMSO (vehicle control) was then added at the indicated doses, and the cells were allowed to grow for 14 days undisturbed. The number of colonies remaining on each plate was counted after fixing and staining with crystal violet solution. SK-CO-1 cells, which carry two copies of mutated *ATM*, are indicated by circles, and LoVo cells that express WT-*ATM* are indicated by squares. Results show the average of three separate experiments with each treatment carried out in triplicate. Error bars show standard deviation. *******P* < 0.001.

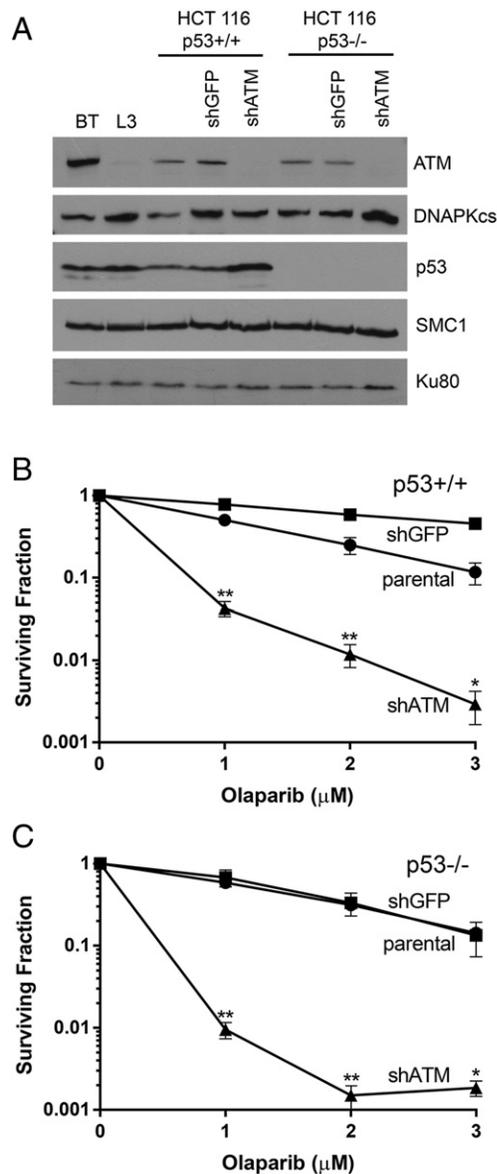


Figure 5. shRNA depletion of ATM in HCT116 cells. (A) ATM was depleted in HCT116 cells using shRNA as described in Materials and Methods. shRNA to GFP was used as a control. Immunoblotting of shRNA control (shGFP) and shRNA ATM is shown. BT and L3 cells were used as ATM-proficient and ATM-deficient controls, respectively. (B) Clonogenic assays with olaparib at 0, 1, 2, or 3 μM with HCT116 p53^{+/+} cells plus and minus shRNA depletion of ATM. HCT116 parental cells are represented by squares, HCT116 shGFP cells by circles, and HCT116 shATM cells by triangles. (C) As in panel B but with HCT116 p53^{-/-} cells. Statistical significance of olaparib sensitivity in shATM depleted cells compared with shGFP control cells is indicated by the asterisks where * = $P < 0.05$ and ** = $P < 0.001$.

We previously showed that MCL and gastric cancer cell lines with loss or low expression of ATM protein are sensitive to the PARP inhibitor olaparib [12–14,17]. Here, we show that ATM-deficient CRC cell lines are also sensitive to olaparib, raising the possibility that PARP inhibitors could have potential in CRC as well as MCL and gastric cancer. Because ATM is required for survival in response to ionizing radiation and other DNA-damaging drugs, we speculate that ATM-deficient CRC cells may also be preferentially sensitized to

radiation and/or chemotherapy, either alone or in combination with a PARP inhibitor.

Genome sequencing results has revealed that up to 18% (13 of 72 patients) of patients with CRC had mutation in ATM (four truncations, nine missense mutations) [38], but the effects of these mutations on ATM function are not known. We show that SK-CO-1 cells, which are homozygous for ATM mutation c2251-10T>G, lack ATM protein expression and are sensitive to olaparib. In contrast, HCT116 cells which are heterozygous for c3380C>T have low levels of ATM protein expression compared with other CRC cells tested yet are still relatively resistant to olaparib unless ATM is further depleted by shRNA or inhibited by KU55933. These results suggest that CRC cells with heterozygous mutations in ATM that retain ATM function may not show sensitivity to PARP inhibitors. Careful analysis of the effects of ATM mutation on ATM function and PARP inhibitor sensitivity is therefore warranted and will be an important factor in determining ATM function and potential for PARP inhibitor therapy in patients.

Our previous studies in MCL and gastric cancer showed that mutation or deletion of p53 enhanced sensitivity to olaparib in cells with loss or depletion of ATM, revealing that p53 status can modulate the response to olaparib in cells with ATM inactivation or deficiency [12–14,17]. ATM-deficient SKCO1 cells were sensitive to olaparib despite the presence of wild-type p53; however, whether p53 is functional in these cells in the absence of functional ATM is not known. To further address the role of p53 in olaparib sensitivity in ATM-deficient cells, we depleted ATM in HCT116 cells that were either wild type for p53 or had p53 deletion. Again, reduction of ATM protein expression resulted in olaparib sensitivity whether p53 was present or not. Similarly, ATM-proficient cells were sensitive to olaparib in the presence of an ATM kinase inhibitor regardless of the presence of p53, although in both cases (shATM depletion and ATM inhibition by KU55933), cells with loss of p53 were more sensitive to olaparib than those with wt p53. It has been reported that over 80% of CRCs have mutation of TP53 [23]. Our results suggest that these cells would be more sensitive to the combination of an ATM inhibitor and a PARP inhibitor than those with wt-p53. Whether normal colorectal cells with wt-p53 are also sensitive to the combination of ATM and PARP inhibitor is an important factor that remains to be determined if PARP inhibitors are used in the treatment of patients with CRC.

Together, these studies suggest that patients with MCL, gastric, CRC, and perhaps other cancers with mutations in ATM that result in loss of protein or function may be sensitive to PARP inhibitors. Indeed, results of recent phase II clinical trials indicate that patients with gastric cancer and low ATM expression showed better overall survival with paclitaxel and olaparib treatment than those with gastric cancer and normal ATM levels [18], and prostate cancer patients with ATM mutation responded well to olaparib [6]. In summary, an increasing number of human cancers have been shown to contain alterations in ATM. As in A-T, these somatic mutations, which are largely either missense or truncating mutations, result in modifications throughout the entire ATM coding region [35], and their precise effect on ATM function and/or expression is generally not known. However, when these mutations compromise ATM function, they hold the potential to confer sensitivity to PARP inhibitors as well as to traditional chemotherapeutic agents. Going forward, it will be important to develop tumor biomarkers not only for ATM function but also for p53 and other modifying factors to maximize opportunities to target ATM-deficient tumors for cancer therapy.

Materials and Methods

Cell Culture

HCT116 cells with wild-type p53 ($p53^{+/+}$) and HCT116 with deletion of p53 ($p53^{-/-}$) cells were cultured in McCoy's 5A medium (Gibco) with 10% (v/v) fetal bovine serum (GE Healthcare, HyClone, Cat SH30396). SK-CO-1 and LS123 were cultured in MEM medium with 10% (v/v) fetal bovine serum, 1% (w/v) nonessential amino acid, and 1% (w/v) sodium pyruvate. SNU-C1 and LoVo cells were cultured in RPMI or F12K medium with 10% of fetal bovine serum, respectively. T84 cells were cultured in DMEM/F12 medium with 5% (v/v) fetal bovine serum. BT and L3 cells were cultured as described previously [17]. All cell lines were cultured at 37°C in the presence of 50 U/ml of penicillin and 50 µg/ml of streptomycin (Gibco) under an atmosphere of 5% CO₂. Olaparib and KU55933 (Selleck Chemicals) were dissolved in DMSO and stored at -80°C.

Transfections

Stable transfection of HCT116 $p53^{+/+}$ and HCT116 $p53^{-/-}$ cell lines (a kind gift from Dr. B. Vogelstein) were performed as described previously [17]. Transfected cells were selected by culturing in medium containing 4 µg/ml of puromycin (Sigma-Aldrich), and single cell colonies were isolated.

Immunoblots

Cells were harvested and lysed by NETN lysis followed by analysis on SDS-PAGE gels [17]. Antibodies to ATM (Upstate, cat # 05-513), pS1981/ATM (Epitomics, cat # 2152-1), pS2056/DNA-PKcs (Abcam, cat # ab18192), Ku80 (Abcam, cat # ab3107), SMC1 (Novus, cat # Nb100-204), p53 (Santa Cruz, cat # SC126), pS15/p53 (Cell Signaling, cat # 9284), and KAP1 (Abcam, cat # ab10483) were purchased commercially. The antibody to pS824/KAP1 was made in-house and used at concentration of 2 µg/ml. Where indicated, cells were either unirradiated or irradiated at 2 Gy using a Gammacell 1000 tissue irradiator (MDS Nordion) with a ¹³⁷Cs source and a dose rate of 2.9 Gy/min and harvested after 1 hour.

Clonogenic Survival Assays

Cells were trypsinized, seeded in 6-cm dishes, and allowed to grow 24 hours before treatment with olaparib at 1, 2, or 3 µM or an equal volume of DMSO (as control). Cells were allowed to grow undisturbed for 14 days, followed by fixing and staining with crystal violet, and the surviving colonies with over 50 cells were counted [17]. Results show the average of at least three experiments with standard deviation. Statistical significance was determined using a two-way analysis of variance, and *P* values less than .05 (indicated by *) were considered statistically significant.

Conflict of Interest

The authors have no conflicts of interest to declare.

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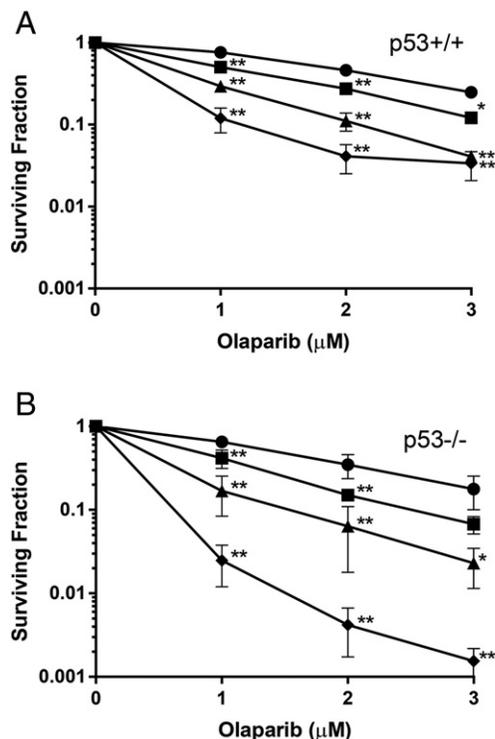


Figure 6. Clonogenic assays in HCT116 $p53^{+/+}$ and HCT116 $p53^{-/-}$ cells treated with the PARP inhibitor olaparib and ATM inhibitor KU55933. (A) HCT116 $p53^{+/+}$ cells were treated with either DMSO (circles) or 2.5 (squares), 5 (triangles), or 7.5 µM (diamonds) of the ATM inhibitor KU55933 in addition to treatment with olaparib at 0, 1, 2, or 3 µM. (B) As in panel B but with HCT116 $p53^{-/-}$ cells. Statistical significance of olaparib sensitivity in shATM depleted cells compared with shGFP control cells is indicated by the asterisks where * = *P* < 0.05 and ** = *P* < 0.001.

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