Published OnlineFirst December 19, 2013; DOI: 10.1158/1535-7163.MCT-13-0749

Molecular Cancer Therapeutics

Glioblastoma Cells Containing Mutations in the Cohesin Component *STAG2* Are Sensitive to PARP Inhibition

Melanie L. Bailey[1], Nigel J. O'Neil[1], Derek M. van Pel[2], David A. Solomon[3], Todd Waldman[4], and Philip Hieter[1]

Abstract

Recent data have identified STAG2, a core subunit of the multifunctional cohesin complex, as a highly recurrently mutated gene in several types of cancer. We sought to identify a therapeutic strategy to selectively target cancer cells harboring inactivating mutations of *STAG2* using two independent pairs of isogenic glioblastoma cell lines containing either an endogenous mutant *STAG2* allele or a wild-type *STAG2* allele restored by homologous recombination. We find that mutations in *STAG2* are associated with significantly increased sensitivity to inhibitors of the DNA repair enzyme PARP. *STAG2*-mutated, PARP-inhibited cells accumulated in G_2 phase and had a higher percentage of micronuclei, fragmented nuclei, and chromatin bridges compared with wild-type *STAG2* cells. We also observed more 53BP1 foci in *STAG2*-mutated glioblastoma cells, suggesting that these cells have defects in DNA repair. Furthermore, cells with mutations in *STAG2* were more sensitive than cells with wild-type *STAG2* when PARP inhibitors were used in combination with DNA-damaging agents. These data suggest that PARP is a potential target for tumors harboring inactivating mutations in *STAG2*, and strongly recommend that STAG2 status be determined and correlated with therapeutic response to PARP inhibitors, both prospectively and retrospectively, in clinical trials. *Mol Cancer Ther; 13(3); 724–32. ©2013 AACR.*

Introduction

Inhibition of PARP has emerged as a promising drug strategy for the treatment of cancers mutated for BRCA1/2 because of its ability to selectively kill cells through synthetic lethality (1, 2). More recently, PARP inhibitors have been shown to be effective in cells with defects in other genes involved in homologous recombination and the DNA damage response suggesting that PARP inhibitors may be effective in treating a wider range of tumors that do not have BRCA mutations (3–6). Identification of other tumor genotypes susceptible to PARP inhibition will expand the utility of these drugs.

The cohesin complex, named for its role in sister chromatid cohesion, is well conserved across organisms (7). In humans, the core mitotic complex consists of four subunits: SMC1A, SMC3, RAD21 (also known as SCC1 or MCD1), and one of two possible stromal antigen proteins (STAG1 or STAG2). Together, these four subunits can encompass newly replicated sister chromatids and hold them in close proximity (8). Beyond its well-known function in chromosome segregation, cohesin has several additional roles in the cell. Similar to other genes sensitive to PARP inhibition, defects in cohesin components affect both replication fork integrity and homologous recombination repair (7, 9, 10). Cohesin is recruited to sites of replication fork pausing and double-strand breaks (DSB) and has also been shown to promote replication fork restart and DNA repair through its interactions with other proteins (11–13). In addition, because of its ability to encircle sister chromatids, the cohesin complex is thought to promote error-free recombination repair with the neighboring undamaged DNA strand in the S–G_2 phases of the cell cycle (10). Supporting the idea that cells mutated for cohesin genes might be sensitive to PARP inhibition, we have shown that knockdown of three of the cohesin core components (SMC1, SMC3, and RAD21) can render cells sensitive to the PARP inhibitor olaparib (14).

Recently, the cohesin gene, *STAG2*, was discovered to be highly mutated in glioblastoma, Ewing sarcoma, and melanoma cells (15). These mutations led to either truncation or functional inactivation of the STAG2 protein that is easily detected in cells or tissues by immunohistochemistry or Western blot analysis using antibodies. Given the previous data that knockdown of cohesin components results in PARP inhibitor sensitivity (14), we wanted to determine if tumor cells with

Authors' Affiliations: [1]Michael Smith Laboratories, University of British Columbia; [2]The Centre for Drug Research and Development, Vancouver, British Columbia, Canada; [3]Department of Pathology, University of California San Francisco, San Francisco, California; and [4]Department of Oncology, Lombardi Comprehensive Cancer Center, Georgetown University School of Medicine, Washington, DC

Corresponding Author: Philip Hieter, Michael Smith Laboratories, 2185 East Mall, Room 323, University of British Columbia, Vancouver, British Columbia, Canada V6T1Z4. Phone: 604-822-5115; Fax: 604-822-2114; E-mail: hieter@msl.ubc.ca

Note: Supplementary data for this article are available at Molecular Cancer Therapeutics Online (http://mct.aacrjournals.org/).

doi: 10.1158/1535-7163.MCT-13-0749

Published OnlineFirst December 19, 2013; DOI: 10.1158/1535-7163.MCT-13-0749

STAG2 mutations were susceptible to PARP inhibition. Here, we show that glioblastoma cell lines with mutations in *STAG2* are significantly more sensitive to PARP inhibitors than matched, isogenic *STAG2* wild-type lines. This proliferation defect results in an accumulation of cells in G_2 phase and genome instability. Furthermore, *STAG2*-mutated cell lines demonstrate an increased sensitivity when combinations of DNA-damaging chemotherapeutics and PARP inhibitors are used, providing a therapeutic rationale for PARP inhibitors either as a single agent, or in combination with other DNA-damaging agents, in *STAG2*-deficient tumors.

Materials and Methods

Materials and cell culture

Olaparib (AZD2281), veliparib (ABT-888), and rucaparib (AG014699) were purchased from Selleck Chemicals; temozolomide and camptothecin were purchased from Sigma-Aldrich. Antibodies used were anti-PAR (Trevigen), anti-STAG2 (Santa Cruz Biotechnology), anti-SMC1, anti-SMC3, anti-pS10 Histone H3 (pH3), anti-53BP1anti-GAPD, and anti-α-tubulin (all from Abcam). H4 and 42MGBA parental and *STAG2* knock-in (KI) cell lines have been described previously (15). H4 and 42MGBA cell lines obtained from Solomon and colleagues were from the American Type Culture Collection and DSMZ, respectively, and were cultured in Dulbecco's Modified Eagle Medium (DMEM) + 10% FBS at 37°C and 5% CO_2 for 1 to 2 months at a time before reinitiation from early passage, frozen stocks. Cell lines were checked regularly for the presence or absence of STAG2 by Western blot analysis (Supplementary Fig. S1).

Cell counting experiments and clonogenic assays

To assess cell number by nuclei counting, cells were plated in a 96-well format with 6 technical replicates for each drug concentration. Twenty-four hours after plating, inhibitors or dimethyl sulfoxide (DMSO) were diluted into DMEM and added to wells. Cells were fixed in 3.7% paraformaldehyde after 4 to 5 days and then stained with Hoechst 33342 before nuclei were counted on a Cellomics Arrayscan VTI.

For clonogenic assays, cells were plated at single-cell density in 6-well dishes with three replicates per drug concentration. Drugs were added after 24 hours and cells were allowed to grow for 10 to 14 days; drug media were changed every 4 to 5 days. Colonies were then fixed and stained with 0.1% crystal violet in 95% ethanol for counting. Cell lines were all normalized to the DMSO control and compared using a two-tailed, unmatched Student *t* test. Error bars represent SEM.

Immunoblotting and flow cytometry

Cells were grown with or without PARP inhibitor for 3 (H4) or 4 (42MGBA) days before all cells were collected by trypsinization and centrifugation. For immunoblotting, pellets were resuspended in 50 mmol/L of Tris-HCl (pH 7.5), 150 mmol/L of NaCl, 1% Triton X-100, and protease inhibitors (Roche). Cells were lysed by sonication and centrifuged to remove debris. Lysates were separated by SDS–PAGE, transferred to polyvinylidene difluoride, and blotted with the indicated antibodies.

For flow cytometry, cells were grown and harvested as above, before being fixed in cold 70% ethanol. Where indicated, cells were first stained with pH3 antibody followed by anti-rabbit conjugated to Alexa Fluor 488 (Jackson Immunoresearch), before being incubated with propidium iodide and RNase A. Cell-cycle analysis was done using FlowJo. Cell lines were compared using a one-tailed, matched Student *t* test. Error bars represent SEM.

Immunofluorescence

Cells were grown on coverslips with and without PARP inhibitor for 3 (H4) or 4 (42MGBA) days before fixation in 1:1 methanol:acetone and permeabilization in 0.1% Triton X-100. Coverslips were incubated with anti-53BP1 and anti-rabbit conjugated to Cy3 (Jackson Immunoresearch) before being stained with 4′,6-diamidino-2-phenylindole (DAPI) and viewed on a Zeiss Axioplan 2 Fluorescence microscope. At least 200 cells were counted for each experiment. For micronuclei, fragmented nuclei, and chromatin bridges, cell lines were compared using a one-tailed, matched Student *t* test. For 53BP1 foci, cell lines were compared using a Fisher exact test.

Results

STAG2-mutated glioblastoma cell lines are sensitive to PARP inhibition

To determine whether *STAG2* mutation causes PARP inhibitor sensitivity, we used two paired sets of glioblastoma cell lines described by Solomon and colleagues (15): H4 (which has a 25-bp insertion in exon 12 of *STAG2*) and 42MGBA (which has a nonsense mutation in exon 20 of *STAG2*), which were each matched with *STAG2* KI lines that have these mutations corrected via HR (H4 *STAG2* KI and 42MGBA *STAG2* KI, respectively). Using these two independent isogenic cell line pairs, we first looked at the proliferation of the H4 and 42MGBA cell lines in the presence of the PARP inhibitor, olaparib, and found that over a range of concentrations, both the H4 and 42MGBA *STAG2*-mutated cell lines showed significantly decreased cell number when compared with their *STAG2* KI counterpart by nuclei-counting (Fig. 1A and B). *STAG2*-mutated cells treated with olaparib also resulted in fewer colonies compared with similarly treated *STAG2* KI cells in clonogenic assays (Fig. 1C; Supplementary Fig. S2A). Finally, when STAG2 was knocked down by short hairpin RNA (shRNA) in HCT116 cells, these cells decreased proliferation in the presence of olaparib similar to the glioblastoma cell lines (Supplementary Fig. S2B and S2C). These results are consistent with our previous findings for siRNA-mediated cohesin knockdown and PARP inhibition (14), and suggest that decreases in cohesin—both the tripartite ring components and the SCC3 ortholog STAG2—sensitize cells to olaparib.

Published OnlineFirst December 19, 2013; DOI: 10.1158/1535-7163.MCT-13-0749

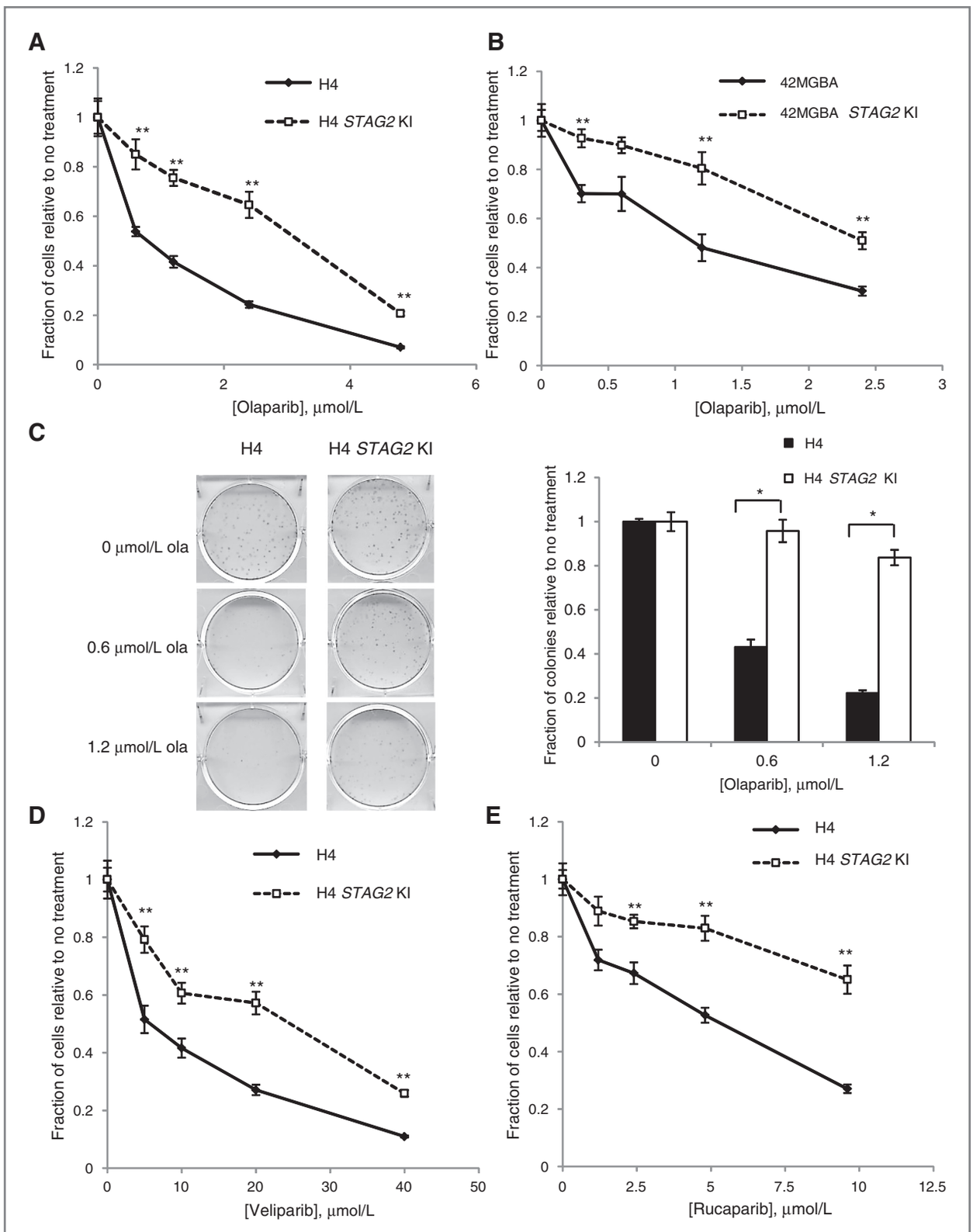

Figure 1. *STAG2*-mutated cell lines are more sensitive to PARP inhibitors. A, *STAG2*-mutated and *STAG2* KI H4 glioblastoma cell lines were treated with increasing concentrations of olaparib in 96-well format and cell nuclei were counted after 4 days. B, 42MGBA glioblastoma cell lines were treated as in A and cell nuclei were counted after 5 days. C, clonogenic survival of H4 cells after olaparib treatment. The graph represents 3 technical replicates. D and E, *STAG2*-mutated and KI cell lines were treated with increasing concentrations of the PARP inhibitors veliparib (D) and rucaparib (E) as in A. **, $P < 0.005$; *, $P < 0.01$.

Published OnlineFirst December 19, 2013; DOI: 10.1158/1535-7163.MCT-13-0749

The cohesin complex contains one subunit each of SMC1, SMC3, and RAD21, as well as one of either STAG1 or STAG2. Excluding STAG2, we noted no difference in the levels of these proteins in the H4 parental and *STAG2* KI cell lines (Supplementary Fig. S1A). Upon immunoprecipitation of RAD21, there was evidence of an increase in STAG1-containing complex in the *STAG2*-mutated line, suggesting compensation in the cells lacking *STAG2* (Supplementary Fig. S1A). Examination of poly ADP-ribose (PAR) levels, a measure of PARP activity, in cells by Western blot analysis showed that PARylation in both the H4 and 42MGBA cell line pairs was high, and this was greatly decreased by treatment with the PARP inhibitor olaparib regardless of *STAG2* status (Supplementary Fig. S1B and S1C). We also analyzed the protein levels of the core cohesin components SMC1, SMC3, and RAD21. As expected, the levels of SMC1, SMC3, and RAD21 were equivalent in untreated H4 *STAG2*-mutated and *STAG2* KI cell lysates (Supplementary Fig. S1D). Interestingly, treatment with olaparib caused a decrease in the protein levels of these core components, and this decrease was much more pronounced in the H4 *STAG2*-mutated cell line. The reason for these low levels is currently not known but suggests that the accessory protein STAG2 stabilizes the cohesin ring components when they are challenged with PARP inhibitor.

To ensure that the loss of survival we observed in *STAG2*-mutated cells was the result of PARP inhibition and not limited to the PARP inhibitor olaparib, we also treated H4- and 42MGBA-paired cell lines with two other PARP inhibitors: veliparib, an oral inhibitor shown to cross the blood–brain barrier (16), and rucaparib, a potent inhibitor which targets a broad spectrum of PARP enzymes (17, 18). Both of these inhibitors were more effective on *STAG2*-mutated cells when compared with *STAG2* KI cells (Fig. 1D and E; Supplementary Fig. S2D and S2E). From these data, we conclude that mutations in the cohesin component *STAG2* render cells more sensitive to PARP inhibition.

PARP inhibition in *STAG2*-mutated cells is associated with an accumulation of cells in G_2 phase and nuclear abnormalities

As a more robust proliferation defect was observed in *STAG2*-mutated cells after treatment with PARP inhibitor, we next sought to determine whether this could be attributed to a specific phase of the cell cycle. Analysis of olaparib-treated H4 cells showed an accumulation of *STAG2*-mutated cells in the G_2–M phase (Fig. 2A and B). Because STAG2-cohesin is involved in cohesion of sister chromatids in both G_2 and mitosis up until their segregation, we also used an antibody to histone H3 phosphorylated at S10 (pH3) as a mitotic marker to differentiate between the mitotic and G_2 cells. Staining with this marker showed very little difference in the percentage of mitotic cells in treated and untreated cells (Fig. 2A), indicating that *STAG2*-mutated cells treated with olaparib accumulate in G_2.

Similarly, 42MGBA *STAG2*-mutated cells treated with olaparib also show an accumulation of cells in G_2–M (Supplementary Fig. S3A and S3B). These results are consistent with H4 cells and suggest that glioblastoma cell lines treated with PARP inhibitor that are deficient for STAG2 show a more prolonged G_2 delay when compared with those that express wild-type STAG2. It should be noted that both H4 and 42MGBA *STAG2* KI cell lines show sensitivity and G_2–M accumulation at higher concentrations of olaparib. As glioblastoma cell lines in general can have multiple mutations and chromosomal abnormalities, it is possible that these lines contain other defects in addition to *STAG2* mutation. However, as growth differences are seen between mutated and KI cells across both H4 and 42MGBA lines, we believe that STAG2 function significantly contributes to PARP inhibitor sensitivity.

Given that we observed a consistent increase in both sub-G_1 and >4N cells when *STAG2*-mutated lines were treated with olaparib (Fig. 2; Supplementary Fig. S3), we next looked for differences in other genome instability and cell death phenotypes. Accordingly, we observed a higher percentage of cells with micronuclei in *STAG2*-mutated, olaparib-treated cells (Fig. 3A; Supplementary Figs. S4, S5, and S6A). We also observed a higher incidence of chromatin bridges in these cells when compared with *STAG2* KI cells (Fig. 3B; Supplementary Fig. S6B). Both of these phenotypes are consistent with these cells having higher genome instability. In addition, both H4 and 42MGBA *STAG2*–mutated cell lines had a higher fraction of fragmented nuclei when treated with PARP inhibitor (Fig. 3C; Supplementary Fig. S6C), which, along with higher percentages of sub-G_1 cells in the flow cytometry profiles, suggests that these cells may be undergoing cell death. As we saw a large percentage of these fragmented nuclei (~12%) in 42MGBA cells treated with olaparib and acknowledged that fragmented nuclei can be a characteristic of apoptosis, we also looked for an increase in the levels of cleaved PARP, an indicator of apoptosis that is downstream of caspase-3/7 activation (19–21), in olaparib-treated cell lines. We did not, however, observe an increase in the levels of cleaved PARP (Supplementary Fig. S1E). Therefore, we believe that the olaparib-mediated cell death is unlikely to be apoptotic.

PARP inhibitor sensitization is characterized by increased levels of DNA damage

As PARP inhibitor-treated cells showed a delay in G_2 phase and eventual genome instability, we hypothesized that these cells may be responding to increased DNA damage. To further examine this, we stained cells for the DNA damage response protein 53BP1, which rapidly forms foci upon DNA damage, and found that more *STAG2*-mutated cells have >5 53BP1 foci after olaparib treatment than similarly treated *STAG2* KI cells (Fig. 3D; Supplementary Fig. 6D). In fact, at 2.4 μmol/L olaparib, the *STAG2*-mutated H4 line had an increase of approximately 10% of cells with >5 53BP1 foci over its *STAG2* KI counterpart (Fig. 3D). This is remarkably similar to the

Published OnlineFirst December 19, 2013; DOI: 10.1158/1535-7163.MCT-13-0749

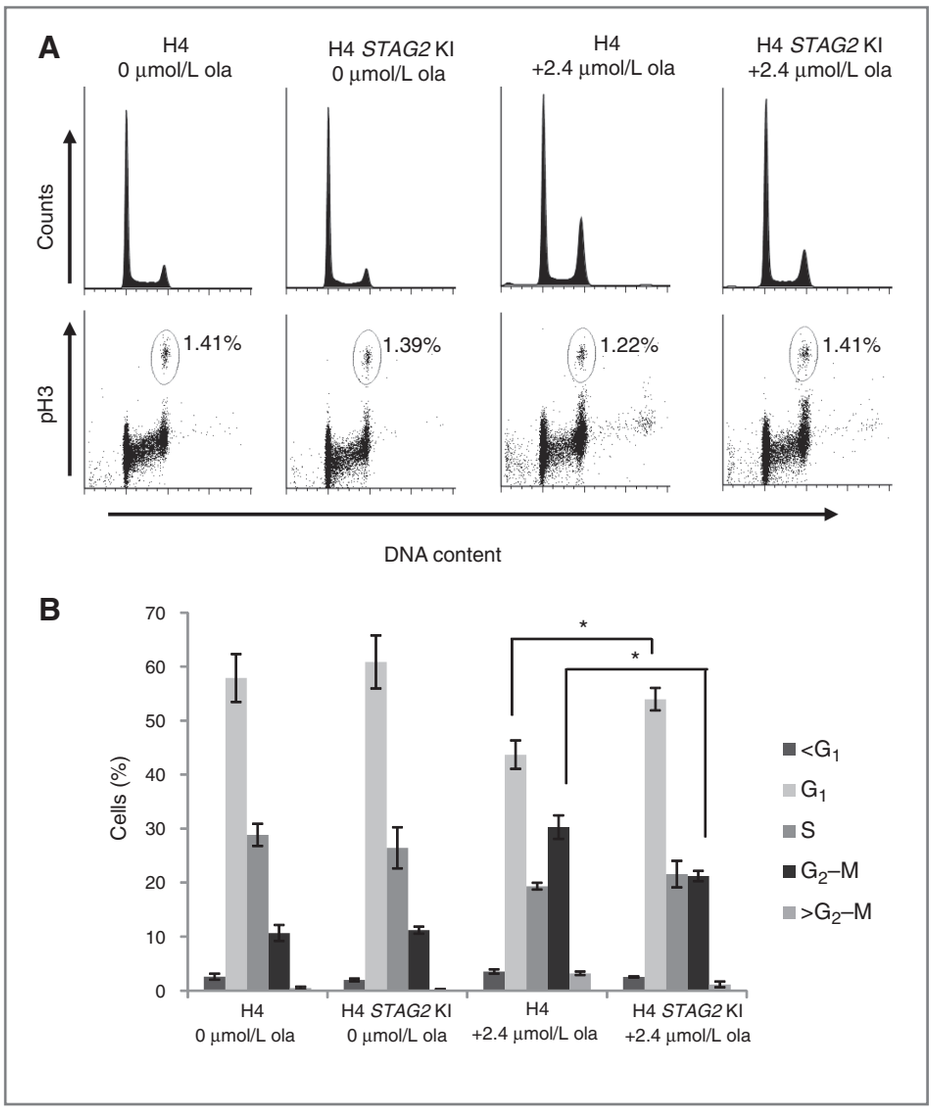

Figure 2. Treatment of *STAG2*-mutated H4 cells with olaparib results in an accumulation of G_2 cells. A, flow cytometry profiles of untreated and olaparib-treated H4 cell lines stained with propidium iodide for DNA content and pS10 Histone H3 antibody as a mitotic marker. B, cell-cycle distribution of H4 cell populations from three independent experiments. *, $P < 0.05$.

approximate 10% increase in G_2 cells seen at the same concentration over the same time period (Fig. 2B). Together, these results suggest that the lower survival rate of *STAG2*-mutated cells after treatment with PARP inhibitor may be due to increased levels of DNA damage, which leads to accumulation of cells in G_2, genome instability, and cell death.

Combining PARP inhibition with camptothecin or temozolomide is more synergistic in *STAG2*-mutated glioblastoma cells

PARP inhibitors have been used extensively to potentiate the toxicity of several chemotherapeutic agents by increasing the DNA damage of these agents (6). Because our paired cell lines showed an increase of 53BP1 foci, a marker for DNA damage, in *STAG2*-mutated cells, we wanted to determine the synergistic effect of PARP inhibition with DNA-damaging agents in *STAG2*-mutated and KI cells. To this end, we tested the effect of the topoisomerase I poison camptothecin, which causes DNA lesions in replicating cells, alone and in combination with olaparib. We found that *STAG2*-mutated H4 cells were more sensitive than H4 *STAG2* KI cells to camptothecin even in the absence of olaparib (Supplementary Fig. S7A). In combination with low doses of olaparib, however, cells were sensitized to a much lower dose of camptothecin than when camptothecin was used alone with a large differential seen between *STAG2*-mutated and *STAG2* KI cells (Fig. 4A). Furthermore, 42MGBA cells were significantly more sensitive than 42MGBA *STAG2* KI cells when treated with both camptothecin and olaparib (Supplementary Fig. S7B).

We also used a combination of olaparib and temozolomide, an alkylating agent that has previously shown robust synergy with PARP inhibitors and is currently used to treat glioblastomas. These two drugs together

Published OnlineFirst December 19, 2013; DOI: 10.1158/1535-7163.MCT-13-0749

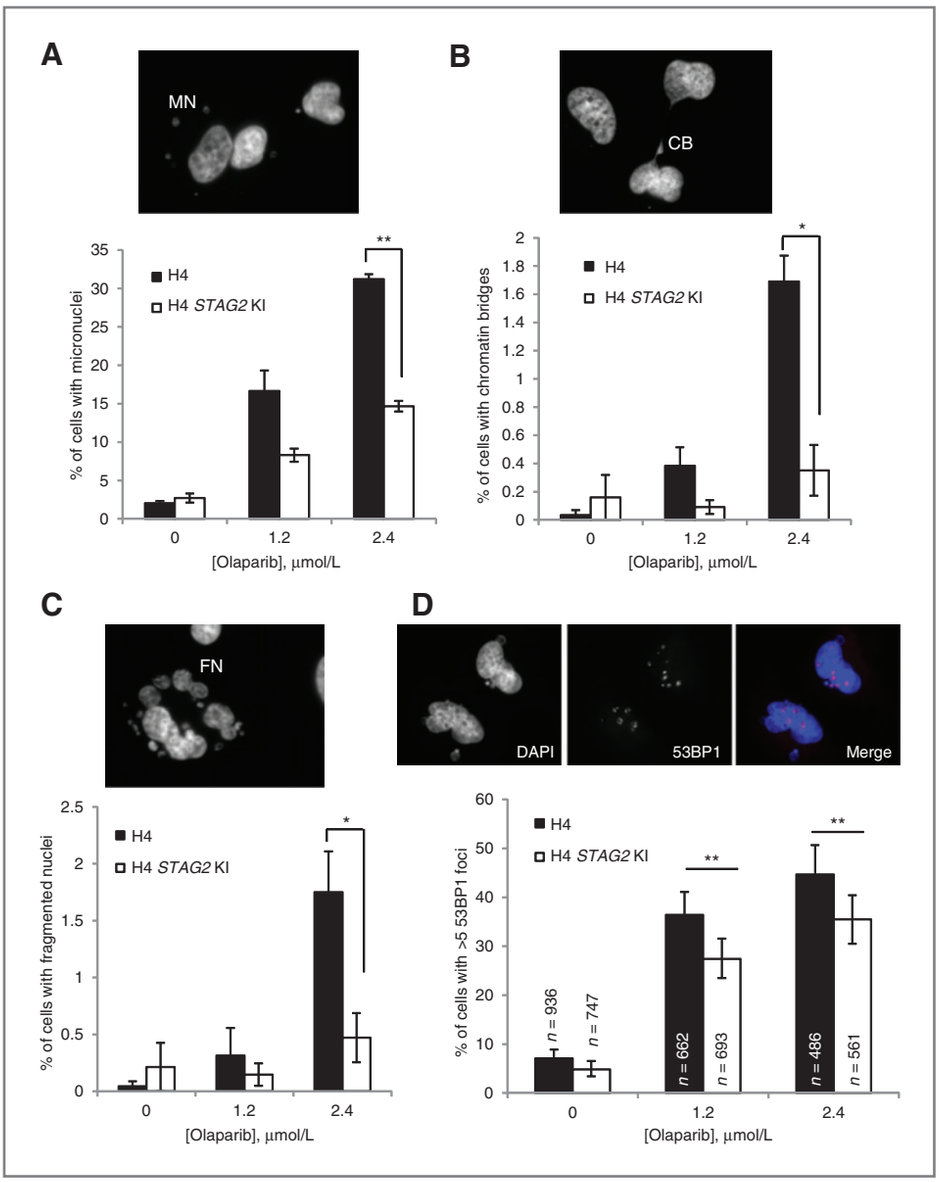

Figure 3. Cells treated with PARP inhibitor show more genome instability and DNA repair defects. A to C, H4 cells grown with or without olaparib for 3 days were scored for the presence of micronuclei (MN, A), chromatin bridges (CB, B), and fragmented nuclei (FN, C). Graphs represent cell counts from three independent experiments. D, presence of 53BP1 foci in paired H4 cell lines untreated and treated with olaparib for 3 days. Error bars in D, 95% confidence intervals. *, $P < 0.05$; **, $P < 0.005$.

had similar results to those with olaparib and camptothecin, showing increased sensitivity in *STAG2*-mutated lines compared with *STAG2* KI lines (Fig. 4B). As both camptothecin and temozolomide are known to be involved in generating lesions that affect DNA replication and repair, our results suggest that the response to these lesions involves not only PARP activity, but also STAG2.

Discussion

The concept of synthetic lethality holds the promise of chemotherapeutics that specifically target tumor cells for killing. Key to the development of synthetic lethal therapeutics is the identification of synthetic lethal interactions between mutations frequently observed in tumors and small molecule inhibitors. PARP inhibitors are a promising class of small molecules that are currently in multiple clinical trials for cancer. The goal of this study was to determine if mutations in the cohesin complex gene *STAG2*, which is frequently mutated in several tumor types and is easily assayed using immunohistochemistry, resulted in sensitivity to PARP inhibitors. In this study, we demonstrated that *STAG2*-mutated glioblastoma cell lines were more sensitive to PARP inhibition than paired cell lines that contained wild-type *STAG2*. This increase in sensitivity in *STAG2*-mutated cells was characterized by increased DNA damage, an accumulation of cells in G_2 phase, and nuclear abnormalities such as chromatin bridges, micronuclei, and fragmented nuclei. PARP inhibition also increased the sensitivity of *STAG2*-mutated cells to the topoisomerase poison camptothecin and the DNA alkylating agent temozolomide, suggesting that

Published OnlineFirst December 19, 2013; DOI: 10.1158/1535-7163.MCT-13-0749

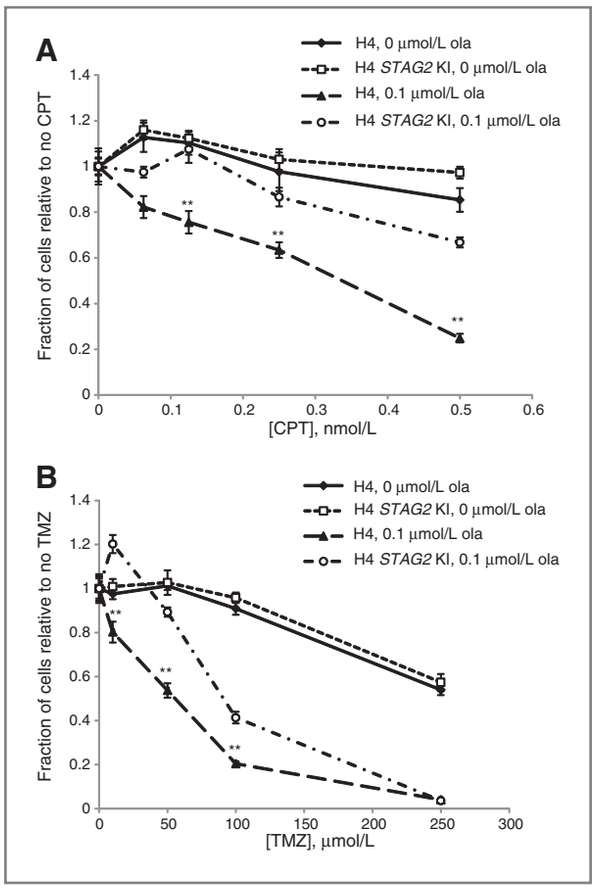

Figure 4. Combinations of PARP inhibitor and known chemotherapeutics are more effective in *STAG2*-mutated H4 cells. Paired H4 cell lines were treated with either camptothecin (CPT, A) and olaparib or temozolomide (TMZ, B) and olaparib and cell number was determined after 4 days using nuclei counting. **, $P < 0.005$.

PARP inhibitors could be used in combination with DNA-damaging agents to cause *STAG2*-mutated tumor cell killing.

Cohesin and cohesin-associated genes are frequently mutated in a number of solid tumor types and leukemias (15, 22–25). More specifically, loss of STAG2 expression has been shown by immunohistochemistry to be common in a significant number of solid tumor types, including glioblastoma (19%), Ewing sarcoma (21%), and melanoma (19%; ref. 15). *STAG2*-truncating mutations have also recently been found in bladder cancer (26–28). A large majority of glioblastoma, melanoma, and Ewing sarcoma tumors had little intratumoral heterogeneity (15), suggesting that sensitivity to PARP inhibitors may be especially relevant in these tumor types.

One explanation for the high frequency loss of STAG2 expression is the location of *STAG2* on the X chromosome, meaning that only a single mutation is needed to inactivate it. Furthermore, unlike the core cohesin components SMC1A, SMC3, and RAD21, which are essential for cell survival, somatic cells have a mitotic STAG2 paralog, STAG1, which may share a level of functional redundancy with STAG2. STAG1 can also form a functional cohesin complex, but unlike STAG2, it has not been found to be lost or mutated in glioblastoma lines (15). The presence of STAG1 may explain why truncating mutations and loss of expression of STAG2 are well tolerated in cells. In support of this, we have found that more STAG1 associates with the cohesin complex in *STAG2*-mutated cells compared with *STAG2* KI cells in immunoprecipitation experiments (Supplementary Fig. S1A). We show here, however, that STAG1 is not sufficient for survival in *STAG2*-mutated cells upon exposure to PARP inhibitors (Fig. 1), and suggest that *STAG2* status in tumors may be a marker for PARP inhibitor sensitivity.

The cohesin complex is multifunctional and has a known role in G_2 DNA repair that has mainly been attributed to its physical ability to hold sister chromatids in close proximity after replication to allow efficient error-free homology searching before recombination or template switching (10, 29). Consistent with DNA repair function, cohesin components have been found to localize to DNA DSBs in human cells (11, 30). Furthermore, Bauerschmidt and colleagues have demonstrated that depletion of SMC1 impairs the repair of radiation-induced DSBs as measured by the increase in γH2AX and 53BP1 foci in G_2 cells (30). Our results show that in the absence of PARP inhibition, *STAG2*-mutated cells have slightly increased 53BP1 foci compared with *STAG2* KI (Fig. 3D; Supplementary Fig. S6D), suggesting that loss of *STAG2* results in increased DNA damage. Inhibition of PARP activity increased the 53BP1 foci differential between the *STAG2*-mutated and KI cells further and also led to the formation of micronuclei and fragmented nuclei (Fig. 3; Supplementary Fig. S6). This suggests that prolonged or less accurate DNA repair in *STAG2*-mutated cells after PARP inhibitor treatment can result in accumulation of cells in G_2 phase, genome instability, or cell death.

Regulation of cohesin dynamics on DNA is controlled by different posttranslational modifications to the complex (31). For instance, both SMC1 and SMC3 are phosphorylated in an ATM-dependent manner and this phosphorylation may be required for efficient mobilization of the complex upon DNA damage (32–34). Similarly, STAG2 is modified by phosphorylation to promote separase-independent dissociation of cohesin from chromosome arms during early mitosis (35), thereby demonstrating that cohesin localization and function can be influenced by STAG2 posttranslational modification. The contribution of STAG2 phosphorylation to the mobilization of cohesin in response to DNA damage, if any, has yet to be determined. In addition to phosphorylation, PARylation by PARP is a posttranslational modification that occurs at sites of DNA damage and is known to affect both chromatin architecture and the recruitment of DNA repair factors (36). It is not known if cohesin is directly PARyled in response to replication stress or DNA damage, but cohesin components have recently been

Published OnlineFirst December 19, 2013; DOI: 10.1158/1535-7163.MCT-13-0749

shown to immunoprecipitate with a PAR antibody after treatment with the DNA alkylating agent, methylnitronitrosoguanidine (MNNG), suggesting an interaction of PAR and cohesin under certain conditions (37). Our results and those of others that have been obtained using PARP inhibitors on cells depleted of cohesin components (14, 38, 39) provide additional evidence of a link between cohesin and PARP activity.

Several chemotherapeutic agents, including temozolomide and camptothecin, are currently in clinical trials with PARP inhibitors as it has been proposed that combining PARP inhibition with DNA-damaging agents will exacerbate their effects (40). Both temozolomide and topoisomerase poisons like camptothecin show increased toxicity in tumors when combined with PARP inhibitors (41–44), and we confirm this synergy in our glioblastoma cell lines (Fig. 4; Supplementary Fig. S7). Several reports have suggested that the potentiation of DNA-damaging agents by PARP inhibition results in an increased need for DSB repair and homologous recombination. For example, combining either temozolomide or camptothecin with PARP inhibitors leads to an increase in DSBs (45, 46). Furthermore, resistance to temozolomide and veliparib in HCT116 cells has been attributed, at least in part, to an increase in Rad51-dependent homologous recombination (47). Other reports have shown that sensitization to alkylating agents by PARP inhibitors is enhanced in cells downregulated for homologous recombination and DSB repair pathway components (48, 49). Our results show that *STAG2* deficiency is another condition that can further sensitize cells to combinations of PARP inhibitors and DNA-damaging agents. Given its mutation or loss of expression in approximately 20% of glioblastomas as well as several other tumor types (15), *STAG2* shows potential as a marker of sensitivity not just to PARP inhibitor monotherapy, but also to combination therapy with camptothecin or temozolomide. Consequently, the status of *STAG2* in tumors should be considered as PARP inhibitors move forward in clinical trials.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: M.L. Bailey, N.J. O'Neil, D.M. van Pel, D.A. Solomon, T. Waldman, P. Hieter
Development of methodology: M.L. Bailey, D.M. van Pel, D.A. Solomon
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M.L. Bailey, D.M. van Pel
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M.L. Bailey, N.J. O'Neil, D.M. van Pel, T. Waldman
Writing, review, and/or revision of the manuscript: M.L. Bailey, N.J. O'Neil, D.M. van Pel, D.A. Solomon, P. Hieter
Study supervision: P. Hieter

Acknowledgments

We would like to thank David Huntsman and members of the Hieter laboratory for helpful discussions during the course of this work. P. Hieter is a Senior Fellow of the Canadian Institute for Advanced Research.

Grant Support

P. Hieter is supported by the NIH (R01CA158162) and the Canadian Institutes of Health Research (MOP-38096). M.L. Bailey is funded by a fellowship from the Michael Smith Foundation for Health Research.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received September 6, 2013; revised December 6, 2013; accepted December 11, 2013; published OnlineFirst December 19, 2013.

References

1. Bryant HE, Schultz N, Thomas HD, Parker KM, Flower D, Lopez E, et al. Specific killing of BRCA2-deficient tumours with inhibitors of poly (ADP-ribose) polymerase. Nature 2005;434:913–7.
2. Farmer H, McCabe N, Lord CJ, Tutt AN, Johnson DA, Richardson TB, et al. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature 2005;434:917–21.
3. Gottipati P, Vischioni B, Schultz N, Solomons J, Bryant HE, Djureinovic T, et al. Poly(ADP-ribose) polymerase is hyperactivated in homologous recombination-defective cells. Cancer Res 2010;70:5389–98.
4. McCabe N, Turner NC, Lord CJ, Kluzek K, Bialkowska A, Swift S, et al. Deficiency in the repair of DNA damage by homologous recombination and sensitivity to poly(ADP-ribose) polymerase inhibition. Cancer Res 2006;66:8109–15.
5. Aguilar-Quesada R, Munoz-Gamez JA, Martin-Oliva D, Peralta A, Valenzuela MT, Matinez-Romero R, et al. Interaction between ATM and PARP-1 in response to DNA damage and sensitization of ATM deficient cells through PARP inhibition. BMC Mol Biol 2007;8:29.
6. Wang X, Weaver DT. The ups and downs of DNA repair biomarkers for PARP inhibitor therapies. Am J Cancer Res 2011;1:301–27.
7. Dorsett D, Strom L. The ancient and evolving roles of cohesin in gene expression and DNA repair. Curr Biol 2012;22:R240–50.
8. Nasmyth K, Haering CH. Cohesin: its roles and mechanisms. Annu Rev Genet 2009;43:525–58.
9. O'Neil NJ, van Pel DM, Hieter P. Synthetic lethality and cancer: cohesin and PARP at the replication fork. Trends Genet 2013;29:290–7.
10. Wu N, Yu H. The smc complexes in DNA damage response. Cell Biosci 2012;2:5.
11. Caron P, Aymard F, Iacovoni JS, Briois S, Canitrot Y, Bugler B, et al. Cohesin protects genes against gammaH2AX induced by DNA double-strand breaks. PLoS Genet 2012;8:e1002460.
12. Strom L, Lindroos HB, Shirahige K, Sjogren C. Postreplicative recruitment of cohesin to double-strand breaks is required for DNA repair. Mol Cell 2004;16:1003–15.
13. Tittel-Elmer M, Lengronne A, Davidson MB, Bacal J, Francois P, Hohl M, et al. Cohesin association to replication sites depends on rad50 and promotes fork restart. Mol Cell 2012;48: 98–108.
14. McLellan JL, O'Neil NJ, Barrett I, Ferree E, van Pel DM, Ushey K, et al. Synthetic lethality of cohesins with PARPs and replication fork mediators. PLoS Genet 2012;8:e1002574.
15. Solomon DA, Kim T, Diaz-Martinez LA, Fair J, Elkahloun AG, Harris BT, et al. Mutational inactivation of STAG2 causes aneuploidy in human cancer. Science 2011;333:1039–43.
16. Donawho CK, Luo Y, Luo Y, Penning TD, Bauch JL, Bouska JJ, et al. ABT-888, an orally active poly(ADP-ribose) polymerase inhibitor that potentiates DNA-damaging agents in preclinical tumor models. Clin Cancer Res 2007;13:2728–37.
17. Thomas HD, Calabrese CR, Batey MA, Canan S, Hostomsky Z, Kyle S, et al. Preclinical selection of a novel poly(ADP-ribose) polymerase inhibitor for clinical trial. Mol Cancer Ther 2007; 6:945–56.

Published OnlineFirst December 19, 2013; DOI: 10.1158/1535-7163.MCT-13-0749

18. Wahlberg E, Karlberg T, Kouznetsova E, Markova N, Macchiarulo A, Thorsell AG, et al. Family-wide chemical profiling and structural analysis of PARP and tankyrase inhibitors. Nat Biotechnol 2012;30:283–8.
19. Duriez PJ, Shah GM. Cleavage of poly(ADP-ribose) polymerase: a sensitive parameter to study cell death. Biochem Cell Biol 1997; 75:337–49.
20. Kaufmann SH, Desnoyers S, Ottaviano Y, Davidson NE, Poirier GG. Specific proteolytic cleavage of poly(ADP-ribose) polymerase: an early marker of chemotherapy-induced apoptosis. Cancer Res 1993;53: 3976–85.
21. Tewari M, Quan LT, O'Rourke K, Desnoyers S, Zeng Z, Beidler DR, et al. Yama/CPP32 beta, a mammalian homolog of CED-3, is a CrmA-inhibitable protease that cleaves the death substrate poly(ADP-ribose) polymerase. Cell 1995;81:801–9.
22. Ding L, Ley TJ, Larson DE, Miller CA, Koboldt DC, Welch JS, et al. Clonal evolution in relapsed acute myeloid leukaemia revealed by whole-genome sequencing. Nature 2012;481:506–10.
23. Welch JS, Ley TJ, Link DC, Miller CA, Larson DE, Koboldt DC, et al. The origin and evolution of mutations in acute myeloid leukemia. Cell 2012;150:264–78.
24. Kon A, Shih LY, Minamino M, Sanada M, Shiraishi Y, Nagata Y, et al. Recurrent mutations in multiple components of the cohesin complex in myeloid neoplasms. Nat Genet 2013;45:1232–7.
25. Cancer Genome Atlas Research Network. Genomic and epigenomic landscapes of adult *de novo* acute myeloid leukemia. N Engl J Med 2013;368:2059–74.
26. Solomon DA, Kim JS, Bondaruk J, Shariat SF, Wang ZF, Elkahloun AG, et al. Frequent truncating mutations of STAG2 in bladder cancer. Nat Genet 2013;45:1428–30.
27. Balbas-Martinez C, Sagrera A, Carrillo-de-Santa-Pau E, Earl J, Marquez M, Vazquez M, et al. Recurrent inactivation of STAG2 in bladder cancer is not associated with aneuploidy. Nat Genet 2013;45:1464–9.
28. Guo G, Sun X, Chen C, Wu S, Huang P, Li Z, et al. Whole-genome and whole-exome sequencing of bladder cancer identifies frequent alterations in genes involved in sister chromatid cohesion and segregation. Nat Genet 2013;45:1459–63.
29. Sjogren C, Nasmyth K. Sister chromatid cohesion is required for postreplicative double-strand break repair in saccharomyces cerevisiae. Curr Biol 2001;11:991–5.
30. Bauerschmidt C, Arrichiello C, Burdak-Rothkamm S, Woodcock M, Hill MA, Stevens DL, et al. Cohesin promotes the repair of ionizing radiation-induced DNA double-strand breaks in replicated chromatin. Nucleic Acids Res 2010;38:477–87.
31. Rudra S, Skibbens RV. Cohesin codes - interpreting chromatin architecture and the many facets of cohesin function. J Cell Sci 2013; 126:31–41.
32. Luo H, Li Y, Mu JJ, Zhang J, Tonaka T, Hamamori Y, et al. Regulation of intra-S phase checkpoint by ionizing radiation (IR)-dependent and IR-independent phosphorylation of SMC3. J Biol Chem 2008;283: 19176–83.
33. Kitagawa R, Bakkenist CJ, McKinnon PJ, Kastan MB. Phosphorylation of SMC1 is a critical downstream event in the ATM-NBS1-BRCA1 pathway. Genes Dev 2004;18:1423–38.
34. Bauerschmidt C, Woodcock M, Stevens DL, Hill MA, Rothkamm K, Helleday T. Cohesin phosphorylation and mobility of SMC1 at ionizing radiation-induced DNA double-strand breaks in human cells. Exp Cell Res 2011;317:330–7.
35. Hauf S, Roitinger E, Koch B, Dittrich CM, Mechtler K, Peters JM. Dissociation of cohesin from chromosome arms and loss of arm cohesion during early mitosis depends on phosphorylation of SA2. PLoS Biol 2005;3:e69.
36. Chou DM, Adamson B, Dephoure NE, Tan X, Nottke AC, Hurov KE, et al. A chromatin localization screen reveals poly (ADP ribose)-regulated recruitment of the repressive polycomb and NuRD complexes to sites of DNA damage. Proc Natl Acad Sci U S A 2010; 107:18475–80.
37. Gagne JP, Pic E, Isabelle M, Krietsch J, Ethier C, Paquet E, et al. Quantitative proteomics profiling of the poly(ADP-ribose)-related response to genotoxic stress. Nucleic Acids Res 2012;40:7788–805.
38. Brough R, Bajrami I, Vatcheva R, Natrajan R, Reis-Filho JS, Lord CJ, et al. APRIN is a cell cycle specific BRCA2-interacting protein required for genome integrity and a predictor of outcome after chemotherapy in breast cancer. EMBO J 2012;31:1160–76.
39. Yadav S, Sehrawat A, Eroglu Z, Somlo G, Hickey R, Yadav S, et al. Role of SMC1 in overcoming drug resistance in triple negative breast cancer. PLoS ONE 2013;8:e64338.
40. Basu B, Sandhu SK, de Bono JS. PARP inhibitors: mechanism of action and their potential role in the prevention and treatment of cancer. Drugs 2012;72:1579–90.
41. Barazzuol L, Jena R, Burnet NG, Meira LB, Jeynes JC, Kirkby KJ, et al. Evaluation of poly (ADP-ribose) polymerase inhibitor ABT-888 combined with radiotherapy and temozolomide in glioblastoma. Radiat Oncol 2013;8:65.
42. Plummer R, Lorigan P, Steven N, Scott L, Middleton MR, Wilson RH, et al. A phase II study of the potent PARP inhibitor, rucaparib (PF-01367338, AG014699), with temozolomide in patients with metastatic melanoma demonstrating evidence of chemopotentiation. Cancer Chemother Pharmacol 2013;71:1191–9.
43. Kummar S, Chen A, Ji J, Zhang Y, Reid JM, Ames M, et al. Phase I study of PARP inhibitor ABT-888 in combination with topotecan in adults with refractory solid tumors and lymphomas. Cancer Res 2011; 71:5626–34.
44. Palma JP, Wang YC, Rodriguez LE, Montgomery D, Ellis PA, Bukofzer G, et al. ABT-888 confers broad *in vivo* activity in combination with temozolomide in diverse tumors. Clin Cancer Res 2009;15:7277–90.
45. Liu X, Shi Y, Guan R, Donawho C, Luo Y, Palma J, et al. Potentiation of temozolomide cytotoxicity by poly(ADP)ribose polymerase inhibitor ABT-888 requires a conversion of single-stranded DNA damages to double-stranded DNA breaks. Mol Cancer Res 2008; 6:1621–9.
46. Zhang YW, Regairaz M, Seiler JA, Agama KK, Doroshow JH, Pommier Y. Poly(ADP-ribose) polymerase and XPF-ERCC1 participate in distinct pathways for the repair of topoisomerase I-induced DNA damage in mammalian cells. Nucleic Acids Res 2011;39: 3607–20.
47. Liu X, Han EK, Anderson M, Shi Y, Semizarov D, Wang G, et al. Acquired resistance to combination treatment with temozolomide and ABT-888 is mediated by both base excision repair and homologous recombination DNA repair pathways. Mol Cancer Res 2009;7: 1686–92.
48. Loser DA, Shibata A, Shibata AK, Woodbine LJ, Jeggo PA, Chalmers AJ. Sensitization to radiation and alkylating agents by inhibitors of poly (ADP-ribose) polymerase is enhanced in cells deficient in DNA double-strand break repair. Mol Cancer Ther 2010;9:1775–87.
49. Quiros S, Roos WP, Kaina B. Rad51 and BRCA2–new molecular targets for sensitizing glioma cells to alkylating anticancer drugs. PLoS ONE 2011;6:e27183.

Published OnlineFirst December 19, 2013; DOI: 10.1158/1535-7163.MCT-13-0749

Molecular Cancer Therapeutics

Glioblastoma Cells Containing Mutations in the Cohesin Component *STAG2* Are Sensitive to PARP Inhibition

Melanie L. Bailey, Nigel J. O'Neil, Derek M. van Pel, et al.

Mol Cancer Ther 2014;13:724-732. Published OnlineFirst December 19, 2013.