PARP1-MGMT complex underpins pathway crosstalk in O6-methylguanine repair

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Abstract
DNA lesions induced by alkylating agents are repaired by two canonical mechanisms, base excision repair dependent on poly(ADP) ribose polymerase 1 (PARP1) and the other mediated by O6-methylguanine (O6meG)-DNA methyltransferase (MGMT) in a single-step catalysis of alkyl-group removal. O6meG is the most cytotoxic and mutagenic lesion among the methyl adducts induced by alkylating agents. Although it can accomplish the dealkylation reaction all by itself as a single protein without associating with other repair proteins, evidence is accumulating that MGMT can form complexes with repair proteins and is highly regulated by a variety of post-translational modifications, such as phosphorylation, ubiquitination, and others. Here, we show that PARP1 and MGMT proteins interact directly in a non-catalytic manner, that MGMT is subject to PARylation by PARP1 after DNA damage, and that the O6meG repair is enhanced upon MGMT PARylation. We provide the first evidence for the direct DNA-independent PARP1-MGMT interaction. Further, PARP1 and MGMT proteins also interact via PARylation of MGMT leading to formation of a novel DNA damage inducible PARP1-MGMT protein complex. This catalytic interaction activates O6meG repair underpinning the functional crosstalk between base excision and MGMT-mediated DNA repair mechanisms. Furthermore, clinically relevant chronic temozolomide exposure induced PARylation of MGMT and increased binding of PARP1 and MGMT to chromatin in cells. Thus, we provide the first mechanistic description of physical interaction between PARP1 and MGMT and their functional cooperation through PARylation for activation of O6meG repair. Hence, the PARP1-MGMT protein complex could be targeted for the development of advanced and more effective cancer therapeutics, particularly for cancers sensitive to PARP1 and MGMT inhibition.

Keywords: DNA damage and repair, Protein interaction, PARP1, MGMT, O6-Methylguanine, Cancer therapy, Ewing sarcoma

To the editor,
Therapeutic synergy induced by PARP1 inhibition combined with DNA alkylation has been reported by several groups [1, 2]. However, we recently demonstrated that despite the antitumor activity in Ewing sarcoma xenografts, half of the tested models were resistant to the combination of talazoparib (PARP1 inhibitor) and temozolomide (standard-of-care DNA alkylating agent) [3]. Exome sequencing analysis revealed no genetic alterations associated with this response. To guide the rational development of more effective cancer therapeutics targeting PARP1 and MGMT mechanisms responsible for repair of alkylation DNA damage, one approach is to understand how cells process DNA lesions [3–5]. It is generally thought that PARP1-mediated base excision repair (BER) and MGMT represent two distinct mechanisms for removing DNA damage induced by temozolomide [6]. In this study, we demonstrate that these
mechanisms are physically coordinated, indicative of functional pathway crosstalk.

To determine cellular response to pharmacologic and genetic ablation of PARP1 and MGMT in the presence of induced DNA damage (temozolomide), cell viability assays were done on Ewing sarcoma cell lines (Fig. 1a–h). We observed that PARP1 and MGMT inhibition (by talazoparib and O6-benzylguanine) (Fig. 1a, b; Additional File 1: Fig. S1a, c, d) or MGMT gene knockdown (by RNAi) (Fig. 1c, e, f; Additional File 1: Fig. S2a, b) induced cell sensitization to temozolomide (up to 20-fold inhibition). We surmise that PARP1 and MGMT may act in a linear pathway of DNA repair in Ewing sarcoma cells and observe no correlation between PARP1-DNA trapping potency and cell sensitization to temozolomide by the two other PARP1 inhibitors, veliparib and olaparib (Additional File 1: Fig. S1b).

To test the conjecture of physical interaction between PARP1 and MGMT underlying the linear cellular response, we used co-immunoprecipitation, pulldown, and microscale thermophoresis (MST) analyses. The amount of co-immunoprecipitating proteins became enhanced in the temozolomide-induced EW-8 cells (Fig. 1i–k; Additional File 1: Fig. S2c–e). Consistent with these data, co-localization of these proteins in temozolomide-treated cells was increased by confocal imaging (Fig. 1l–n; Additional File 1: Fig. S2f). Similarly, SDS-PAGE and silver staining of the immunoprecipitates from purified recombinant PARP1 and MGMT proteins revealed the direct interaction between N-terminal PARP1 (aa 1–662) and MGMT proteins (Fig. 1o–q). MST yielded a $K_D$ of 165 nM, reflecting a strong purified PARP1 and MGMT affinity (Fig. 1r).

We next asked whether PARP1 can PARylate MGMT, and whether this is one of the interaction mechanisms for these proteins. Total cellular PAR levels were determined by ELISA, and PARylation activity of purified PARP1 was analyzed using synthetic single- and double-strand DNA probes with/without O6meG damage, and in the presence/absence of NAD$. Importantly, MGMT was PARylated by PARP1, and the strongest increase in MGMT PARylation was observed in the presence of a double-strand DNA-O6meG oligo (lanes 9 & 22; consistent with PARP1 auto-modification activation) (Fig. 2a, b; Additional File 1: Fig. S3a, b). In the cellular context, the total PAR signal measured by ELISA was induced by temozolomide treatment (Fig. 2c).
Fig. 1 (See legend on previous page.)
To elucidate the significance of MGMT PARylation, the MGMT repair activity was analyzed using PvuII restriction digestion in the presence of NAD⁺-dependent 32P-labeled-O6meG-dsDNA probe and PARP1. MGMT PARylation led to significant NAD⁺-dependent enhancement of O6meG repair indicating that PARylation-mediated PARP1-MGMT complex is formed to increase DNA repair (Fig. 2d–f; Additional File 1: Fig. S3c, d). Further, PARylation in EW-8 cells was measured by immunoblotting using short-term (2 mM, 2 h) and more clinically relevant ‘chronic’ (100 μM, 72 h) temozolomide treatment, which induced PARylation and MGMT signals at 100 μM (Fig. 2g–h). Further, temozolomide can stabilize MGMT levels in the global transcription inhibition context (Additional File 1: Fig. S3e) suggesting that de novo MGMT translation does not take place in response to DNA damage. To ascertain whether MGMT PARylation leads to protein stabilization or enhances association with chromatin and/or PARP1, the identification of PARylation sites on MGMT, generation of MGMT mutants that are refractory to PARylation, and extensive analyses of the effect of these mutations on the basal attributes of MGMT is required. Furthermore, the subcellular protein fractionation showed PARP1 and MGMT binding to chromatin under extended temozolomide treatment as reported by others for co-immunoprecipitated glioblastoma cell lysates (Fig. 2i; Additional File 1: Fig. S3f–g) [7]. It is plausible that in glioblastoma cells the sensitization to PARP1 inhibition is linked to BER impairment rather than MGMT activity. In MGMT-deficient gliomas, the DNA mismatch repair can be activated providing an alternative mechanism to O6meG repair and cell survival. Consistent with our cell-free data, the fractionation results suggest that temozolomide induces PARP1 and MGMT binding to chromatin, where MGMT responds to clinically relevant ‘chronic’ drug exposure. Finally, we verified that PARP1 and MGMT form a complex in several other cell lines, including rhabdomyosarcoma, rhabdoid tumor, synovial sarcoma, and fibroblasts, indicating that this interaction is not cell-type specific (Additional File 1: Fig. S1e–g).

In summary, we present the first evidence of the direct crosstalk between PARP1 (via BER) and MGMT, which were previously thought to function independently (Additional File 1: Fig. S1h). We showed that PARP1 and MGMT can use either a non-catalytic (DNA-independent) or catalytic (DNA damage-dependent) mechanism of interaction, and the latter increases O6meG repair activity through PARP1-mediated MGMT PARylation. Cellular levels of the PARylated MGMT and the MGMT bound to chromatin are enhanced by the clinically relevant ‘chronic’ temozolomide exposure suggesting the PARP1-MGMT-mediated DNA repair takes place during the extended cycles of chemotherapies. Finally, many cancer types and neurodegenerative disorders are dependent on PARP1- and MGMT-mediated repair mechanisms, so our findings provide the rationale to consider the PARP1-MGMT complex as a novel therapeutic target for such diseases.
Fig. 2 (See legend on previous page.)
Abbreviations
BER: Base excision repair; BRCA: Breast cancer gene; Co-IP: Co-immunoprecipitation; dsDNA: Double-stranded DNA; ELISA: Enzyme-linked immunosorbent assay; In silico: By means of computer simulation; In vitro: Using purified proteins; IN: In cellular context; MGMT: O6-methylguanine-DNA methyltransferase; MST: Microscale thermophoresis; N, N'-methylguanines; NmethylA; N3-methyladenine; OmeG; O6-methylguanine; NAD: Nicotinamide adenine dinucleotide; PAR: Poly(ADP ribose); PARP1: Poly(ADP ribose) polymerase; PARylation: Poly(ADP ribosyl)ation; SDS-PAGE: Sodium dodecyl sulfate–polyacrylamide gel electrophoresis; ssDNA: Single-stranded DNA; TLZ: Talazoparib; TMZ: Temozolomide.

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s13045-022-01367-4.

Additional file 1. Supplementary Figures S1, S2, S3, and Methods.

Acknowledgements
We thank our colleagues Patrick Sung, Peter Houghton, and Alan Ashworth for guidance and critical reading of the manuscript; John Pascal for sharing the N-terminus PARP1 construct; Yuzuru Shio for providing Aska cells; Dinoah Leyva at NanoTemper for assistance with the MST analysis; the GCCRI High Throughput Screening facility (Matthew Hart) for RNAi screen; and Fuyang Li, Meagan Shinn, and Dylan Palmer for technical assistance.

Author contributions
RK conceived the research; RK, YK, AR designed the experiments; DA, KB, JC, JG, BH, RL, DP, AR, MS performed the experiments; YC, RK, YK, and AR analyzed the data; RK wrote the manuscript; and DA, KB, JC, JC, JG, BH, RL, DP, AR, MS prepared the figures. All authors read and approved the final manuscript.

Funding
This study was supported in part by RP160716 from the Cancer Prevention and Research Institute of Texas (CPRT; to Peter Houghton and Raushan Kurmasheva), P01 CA165905-03 from the National Cancer Institute (NCI) (to Peter Houghton), U01 CA263981-01 (NCI) (to Raushan Kurmasheva and Peter Houghton), R15 CA241801 (NCI), RP160487, and RP190385 (CPRT) (to Patrick Sung), Owens Medical Research Foundation and R50 CA265315 (to Youngho Kurmasheva), Childhood Cancer Research Fund (CCRF), Helen Freeborn Kerr Charitable Foundation, Cure Childhood Cancer, and Greehey Children's Cancer Research Institute (GCCRI) (to Raushan Kurmasheva), and by the RP160732 (CPRT) (to Yidong Chen).

Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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