Alterations in PARP1 dynamics induced by PARP inhibitors in living cells

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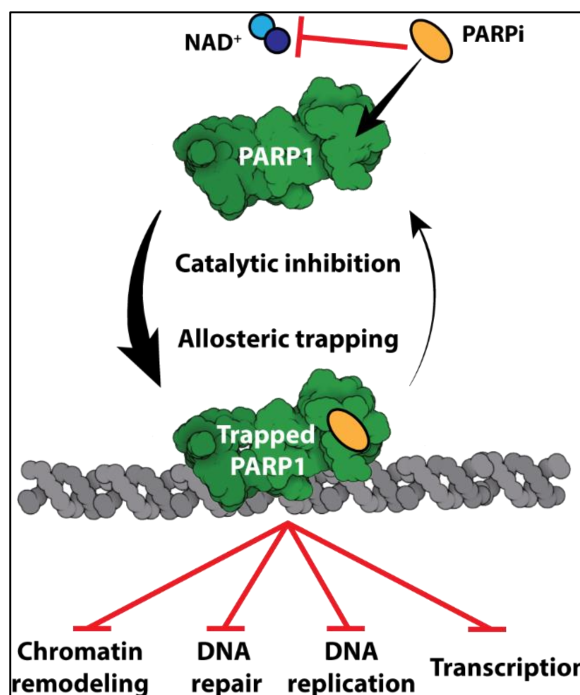
Eukaryotic cells suffer tens of thousands of DNA lesions on a daily basis. The timely repair of these lesions is necessary for preserving genome stability and evading cell transformation and cell death. To this end, cells have evolved an impressive array of DNA repair proteins organized in lesion-specific pathways that deal with particular insults to DNA integrity. Poly(ADP-ribose) polymerase 1 (PARP1) is a crucial DNA repair factor acting as a DNA damage sensor that binds to single- and double-strand DNA breaks to initiate their repair. Binding to DNA breaks triggers the enzymatic activity of PARP1 resulting in the synthesis of long, branched, and negatively charged poly ADP-ribose (PAR) chains that are attached to chromatin proteins near DNA breaks, as well as on PARP1, and which serve as a molecular scaffold for the recruitment of downstream DNA repair proteins. Due to its fundamental role in DNA repair, small molecule inhibitors of PARP1 (PARPi) have entered the clinic for the treatment of various malignancies, most notably homologous recombination (HR)-deficient types of breast and ovarian cancer. PARPi not only inhibit PARP1, but also enhance its association to damaged chromatin, an effect often described as “PARP trapping”. However, how catalytic inhibition and PARP trapping jointly define the potency of various PARPi compounds is currently unknown.

We combined live-cell imaging, UV laser micro-irradiation, fluorescence recovery after photobleaching (FRAP), and mathematical modeling to investigate the effects of seven different PARPi on PARP1 behavior in living cells. We introduce three novel measures of PARP1 dynamics and activity *in vivo*, which facilitated our analysis and the emergence of a universal model for the mechanism of action of PARPi. Based on our results, we propose that both enzymatic inhibition and allosteric trapping of PARP1 contribute to its prolonged association to damaged chromatin, albeit in a strictly independent manner. While catalytic inhibitions causes multiple binding-dissociation cycles of inactive PARP1 molecules, allosteric trapping prolongs the DNA lesion-bound state of PARP1 to largely increase its overall retention at damaged chromatin. Most notably, the overall retention of PARP1 correlates strongly with the delay in the recruitment of downstream DNA repair factors and PARPi cytotoxicity in our model system. Hence, we propose PARP1 retention elicited by PARPi as the major clinically relevant biomarker for PARPi efficacy. Our live-cell imaging approach could speed up the discovery and pre-clinical evaluation of novel PARPi compounds, as well as the emergence of various other DNA repair-targeting drugs to deliver better mechanism-driven therapeutic solutions for cancer patients.



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