The Effect of Current PARP Inhibitors on PARylation of Substrates

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Abstract

The DNA damage response (DDR) is a complex and sophisticated process that works to maintain genomic integrity. Genomic instability is a leading attribute of cancer and can result from mutated components of the DDR. PARP1 and PARP2, initiating proteins in the DDR pathway, have DNA binding sites as well as catalytic domains that allow for the process of PARylation to take place. PARylation is the addition of ADP-ribose chains to certain amino acids. Essentially, this creates a scaffold in order for more protein binding to occur. Throughout this extensive process, the breaks on the DNA are repaired. This makes the process of PARylation during DDR a crucial target when it comes to cancer therapeutics. With this in mind, it would be extremely beneficial to identify the effect that PARP inhibitors have on the PARylation step of the DNA damage response. By using the RuleBender platform, we developed a pharmacodynamic, mechanistic computational model to evaluate the effect of present-day PARP inhibitors on the PARylation activity of PARP substrates, specifically XRCC1. We focus on the double strand break repair process while analyzing the relationship it has with respect to inhibitor selectivity of the molecules PARP1 and PARP2. Both selective and non-selective FDA approved agents were evaluated, and we confirmed that application of these inhibitors within the model reduced PARylation of XRCC1 to varying degrees. Thus, we were able to provide insight into the kinetic activity of the enzymes involved in the PARylation process of the DDR.

Introduction

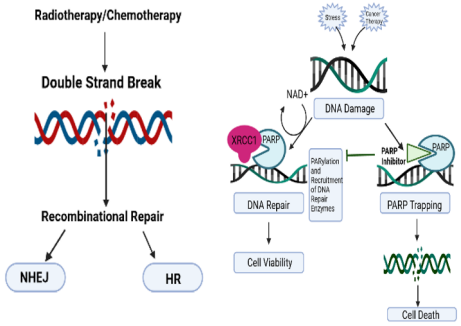


Figure 1. When DNA Double Strand Break (DSB) occurs, there are two main pathways that can be taken. One involves repair through a mechanism referred to as Non-Homologous End Joining (NHEJ) and the other being Homologous Repair (HR). The involvement of the PARP family, specifically PARP1 and PARP2, have yet to be fully understood.

Figure 2. PARP Inhibitors are a type of targeted therapy in cancer treatment. When the DDR is exposed to PARP inhibitors, the binding site on NAD+ is blocked simultaneously with the blocking of the PARylation process. This can happen as long as the inhibitor is bound to the NAD+ site. This stops the dissociation of PARP from the damaged site and may eventually lead to PARP trapping.

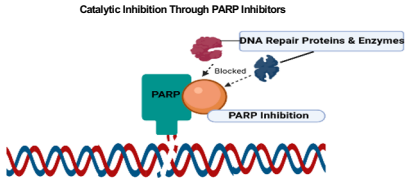
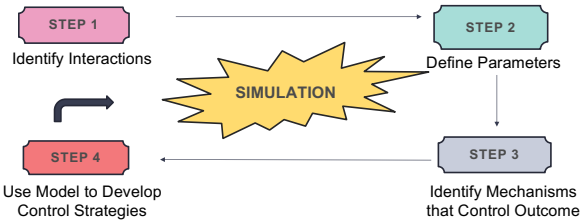


Figure 3. "Catalytic Inhibition" ; Prevents PARP mediated DNA Damage Repair Pathway (DDRP); When the inhibitor binds to the enzyme and interacts, it creates a decrease in the enzymes ability to have its full catalytic effect.

Methods



Parameters

DRUG	PARP1 Ki Value (nM)	PARP2 Ki Value (nM)
Niraparib	3.2	4.0
Talazoparib	1.2	0.9
Rucaparib	1.4	Reported PARP1 Selectivity
Olaparib	Not Found	Not Found

Contact Map

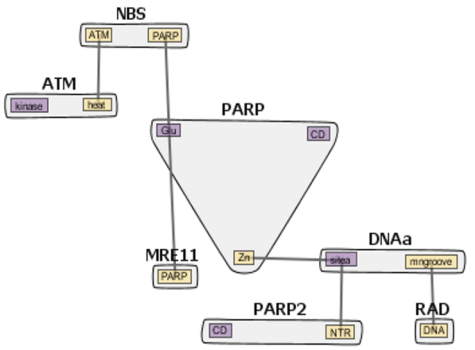
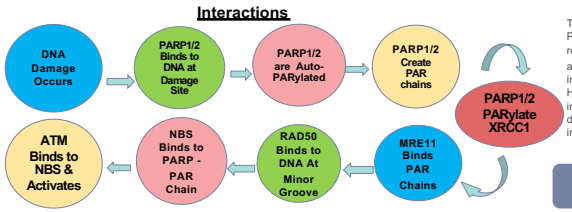
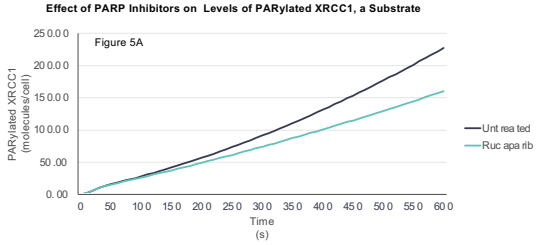
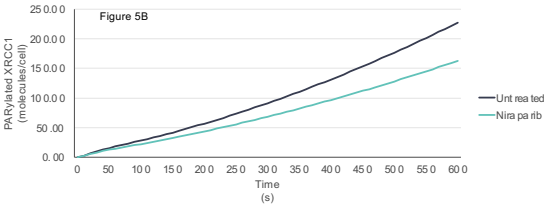


Figure 4. PARP1 binds to DNA at the site of damage through its zinc finger domains, specifically the N-terminus. This causes the molecule to go through an auto-modification process called auto-PARylation, creating PAR polymers on itself. Once this takes place, PARP1 is fully activated and will begin PARylating varying substrates, including MRE11 and XRCC1. PARP2 participates in a series of events similar to that of PARP- and eventually creates PAR chains as well. Once MRE11 and NBS bind to the new chain, RAD50 binds to the minor groove site of DNA, ATM binds to the NBS C-terminus, activating it and again, starting a series of events through phosphorylation of downstream cell cycle checkpoints and other effectors.



Results





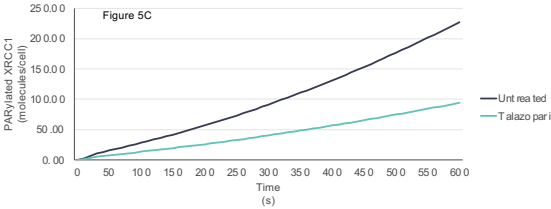


Figure 5. Comparison of effect of PARP inhibitors on the kinetic activity of the PARylation on substrate XRCC1 by PARP1 and PARP2 molecules. Fig. 5A indicates that at 600s, PARylated XRCC1concentration level is reduced by Rucaparib by 30%. When treated with a PARP inhibitor Niraparib (Fig. 5B), PARyated XRCC1 is reduced 29% In Fig.5C, Talazoparib treatment reduced PARylated XRCC1 by 58%.

Conclusion

The addition of a PARP inhibitor does impact the concentration level of PARylated XRCC1 during the DNA damage response, indicating a relationship between PARP inhibition and enzyme-substrate PARylation activity. Based on the preliminary results, the non-selective PARP inhibitor, Talazoparib, reduced substrate PARylation most effectively. However, both the nonselective (Niraparib) and selective (Rucaparib) inhibited substrate PARylation equally. Thus, substrate PARylation is dependent on the Ki value of non-selective and selective PARP inhibitors.

Future Directions

In the future, it is suggested that further analysis be done on the kinetic effect of PARP Inhibitors on the PARylation of the PARP molecule and other involved substrates during the DNA damage response (DDR). Looking into the consequences of inhibition on the recruitment of different complexes, enzymes, or substrates could deem to be vital in identifying key players in the therapeutic impacts that PARP inhibitors have on patients. Key players such as BRCA1, Histone 2AX, CtIP, etc. may allow for more control on the activity of the inhibitors as compared to the tested substrate XRCC1. Furthermore, using this model as a base testing ground, one can determine more ideal Ki values in order to possibly eliminate the anemic response that patients have to PARP inhibitors.

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