Design of a Small Molecule Screening Assay to Detect DNA Trapping of PARP1/2

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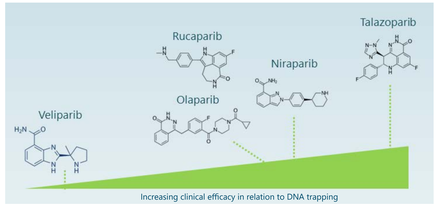
Abstract 6111

ABSTRACT

Poly (ADP-ribose) polymerase (PARP) inhibitors are currently used in the clinic for the treatment of tumors with a defective DNA damage response. When PARP1 or PARP2 binds to damaged DNA, it adds poly (ADP-ribose) chains to itself and to other damage-sensing or repair proteins, while recruiting and activating them. PARylated PARP1/2 next detaches from the DNA so that the other PARylated proteins can initiate the repair process. It has been observed that some PARP inhibitors prevent PARP1/2 from dissociating the DNA. The continuous presence of PARP at the site of damage prevents repair and blocks replication, leading to cell death. Therefore, drugs that trap PARP1/2 to the DNA tend to be significantly more cytotoxic than other PARP inhibitors, which is highly desirable. The ability of an inhibitor to trap PARP to the DNA is directly correlated to its clinical efficacy.

This study describes the design and optimization of novel PARPtrap assays to specifically assess the ability of a drug to trap PARP onto DNA. Proof-of-principle titration of known PARP-trapping inhibitors (Talazoparib, AZD5305, Olaparib and Veliparib) was performed to validate the assay.



The use of these assays indicated that:

i) The known relative trapping efficacies of Talazoparib, Olaparib and Veliparib were similar to known relative efficacies.

ii) Talazoparib, Olaparib and Veliparib had similar trapping efficacy against PARP1 and PARP2, as measured by their EC_{50}. On the other hand, AZD5305 was 1,000 times more efficient at trapping PARP1 than PARP2, demonstrating selectivity between the two proteins.

iii) AZD5305 displayed as efficient DNA trapping activity toward PARP1 as best-in-class Talazoparib.

In summary, we have designed an innovative PARPtrap assay designed for the high throughput screening of small molecule libraries to specifically identify or compare inhibitors that are capable of trapping PARP1 and/or PARP2 onto DNA.

BACKGROUND & ASSAY PRINCIPLE

Maintenance of genome integrity is critical for proper cellular functioning. In humans, over 150 proteins form an intricate DNA damage response network that constantly scans and repairs DNA. The PARP (Poly ADP-Ribose Polymerase) protein family consists of 17 members, which catalyze the ADP-ribosylation of proteins. PARPs are involved in a wide range of biological functions: repair of DNA damage, genome stability, chromatin remodeling, mitotic spindle assembly, regulation of RNA turnover and of gene expression, and DNA methylation.

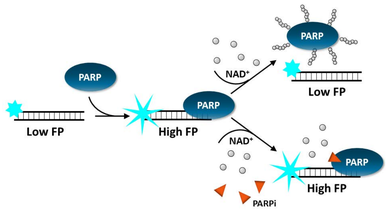
PARP1 and PARP2 are mainly involved in DNA repair, and both proteins regulate this network. PARP2 also regulates epigenetic, proliferative, and inflammatory processes and is important for spermatogonia, thymus, and adipose tissue development. In contrast, PARP1 is a first responder to damaged DNA. It regulates gene transcription and induces apoptosis when DNA is damaged beyond repair.

Some inhibitors prevent the PARylation of PARP and force the protein to remain on the DNA, a phenomenom termed DNA trapping. Since only three members of the PARP family (PARP1 to 3) are DNA-dependent, this trapping is not observed with other PARP family members.

Assay Principle

The assay is based on principles of fluorescence polarization and uses fluorescently labeled DNA probes that are excited by polarized light and emit light with a degree of polarization that is proportional to the rate of molecular rotation.

The free DNA probes that rotate fast have low fluorescence polarization (FP), but high FP when are bound to PARP1 or PARP2. When NAD^+ is added, the PARylated enzymes detach from the probe, reducing FP levels. In the presence of an inhibitor, the inhibitor's trapping ability increases FP in a dose-dependent manner.



RESULTS

Methods

Experiments were performed in duplicates in black, low-binding assay plates. The purified, active enzymes used in this study were GST-tagged PARP1 and PARP2 (BPS Bioscience #80501 and #80502). The proteins were thawed on ice and mixed gently. PARP1 was diluted to 10 ng/reaction and PARP2 was diluted to 75 ng/reaction. Serial dilutions of inhibitors AZD5305 (MedChemExpress), Olaparib (LC Laboratories), Talazoparib and Veliparib (Selleckchem), were prepared in dimethyl sulfoxide (DMSO). The final concentration of DMSO in the assay was kept constant at 1% and was also included in all the controls. PARP enzymes were pre-incubated with the test inhibitors in the presence of PARP-optimized fluorescent-conjugated DNA probes (0.25 nM final in PARP2 assay and 0.5 nM final in PARP1 assay), in the corresponding assay buffer for 30-60 minutes at room temperature. The ribosylation reaction was initiated by adding 10x NAD^+ (except in the "High FP control"). After 60 minutes at room temperature, the fluorescent polarization signal was read using an FP-capable Bio-Tek fluorescent microplate reader using the Blank subtraction setting (excitation 485 nm, emission 530 nm).

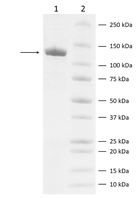


Figure 1: The purity of the recombinant PARP1 protein (BPS Bioscience #80501) was assessed by SDS-PAGE electrophoresis followed by Coomassie blue staining. Lane 1 shows PARP1 and lane 2 shows the molecular weight standards.

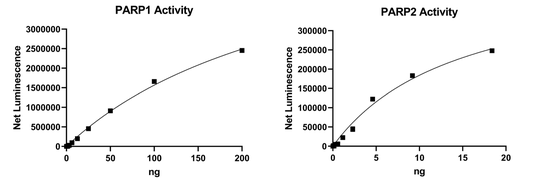


Figure 2: Purified PARP1 and PARP2 enzymatic activity was assessed using a chemiluminescent ELISA (BPS Bioscience #80551 and #80552, respectively). Increasing amounts of PARP proteins were incubated with biotinylated NAD^+ and an optimized activated DNA template on a histone-coated white assay plate. After washing in 0.05% Tween-20-containing phosphate buffer saline, streptavidin-HRP (horseradish peroxidase) was added to the plate. The plate was washed again and a chemiluminescent HRP substrate was added for detection of histone PARylation.

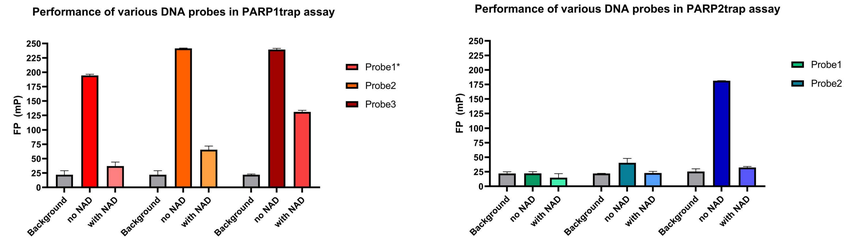


Figure 3: Probe optimization. Various probes specific for PARP1 or PARP2 were assayed in parallel in optimized buffer conditions with pre-determined amounts of PARP proteins as described in Methods. Each probe was assayed with or without NAD^+ to determine the range of signal. Results are shown as raw signal (mP). Results obtained with PARP1 are shown on the left, with PARP2 shown on the right.

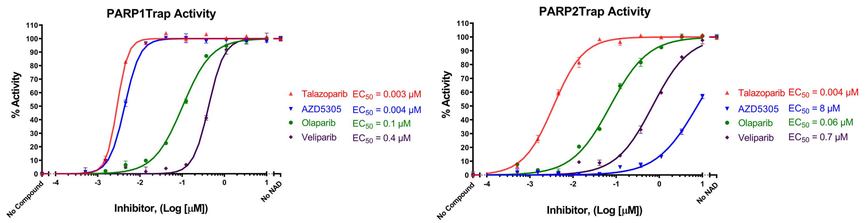


Figure 5: PARP1/DNA trapping (left graph) and PARP2/DNA trapping (right) were measured in the presence of increasing concentrations of Talazoparib, Olaparib, Veliparib, and AZD5305 using the PARPtrap™ Assay Kit. "No compound" corresponds to the "Low FP control" and "no NAD^+" corresponds to the "High FP control". Efficacy, as measured by EC_{50} value, is indicated for each drug on the corresponding graph.

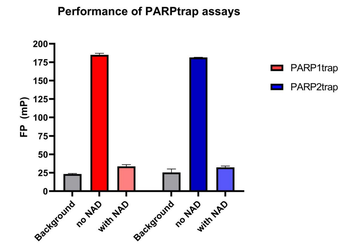


Figure 4: The PARPtrap assay was performed as descibed in Methods to assess the fluorescence polarization signal of reagents alone (background). The difference in FP signal between the reaction performed in the absence of NAD^+ (highest signal allowed by the assay) and in the presence of NAD^+ (lowest signal allowed by the assay) indicates the range of the assay. Results are shown as raw signal (mP).

CONCLUSIONS

Fluorescent probes were optimized specifically for PARP1 and PARP2 trapping. Both assays displayed similar range and sensitivity. Talazoparib, Olaparib and Veliparib had similar trapping efficacy against PARP1 and PARP2, as measured by their EC_{50}, whereas AZD5305 was 1,000 times more efficient at trapping PARP1 than it was PARP2, demonstrating selectivity between PARP1 and PARP2.

The known relative trapping efficacies observed in the assay for Talazoparib, Olaparib and Veliparib were similar to known relative clinical efficacies. AZD5305 displayed as efficient DNA trapping activity toward PARP1 as the current best-in-class Talazoparib.

In conclusion, we have developed an innovative PARPtrap assay designed for the high throughput screening of small molecule libraries to identify or compare inhibitors that are capable of trapping PARP1 and/or PARP2 onto specific DNA probes.