Abstract #5489

Quantitative Intracellular Immunoassay for Monitoring Poly(ADP-Ribose) in Cancer Cells Treated with PARP Inhibitors

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1 Abstract

Poly (ADP-ribose) polymerase (PARP) enzymes play a crucial role in cellular DNA repair mechanisms by catalyzing the addition of poly (ADP-ribose) (PAR) chains to target proteins in response to DNA damage. Overactivity of PARP has been implicated in various pathological conditions, including cancer, where PARP inhibitors (PARPi) have emerged as an important therapeutic strategy. Monitoring PAR levels within different cellular environments is essential for understanding PARP activity and optimizing PARP inhibitor efficacy. This study outlines the characterization and development of a sensitive electrochemiluminescence immunoassay designed to quantitatively measure PAR.

The R-PLEX® PAR Assay was developed on MSD's MULTI-ARRAY® platform. Multiple anti-PAR antibodies were tested in all possible capture/detection combinations using a wide range of PAR polymer concentrations. An optimal pair was selected based on response linearity and sensitivity. To demonstrate the functional application of the PAR immunoassay, three cell lines were cultured under standard conditions in 96-well plates and treated with varying concentrations (0.01–100 µM) of three PARP inhibitors over two time points: 24 or 72 hours. Cell lysates were harvested, protein was quantified, and lysates were added to the PAR immunoassay to determine intracellular PAR levels. IC_{50} curves were generated to evaluate each PARPi's potency.

The R-PLEX PAR Assay demonstrated good sensitivity with an LLOD of 1.82 pM and reliable quantitation of PAR at 0.1 µg lysate per well. The functional assays demonstrated time- and dose-dependent modulation of PAR levels across all cell lines following PARP inhibition. We identified differential IC_{50} concentrations of PARP inhibitors in the PC3 cells within 72 hours demonstrating the utility and throughput of the PAR assay.

This study demonstrates the utility of a novel immunoassay for accurately quantifying PAR in various cancer cell lines subjected to PARP inhibition. This assay offers a robust tool for evaluating PARP inhibitor efficacy and can serve as a valuable asset for researchers in drug development and cellular biology to explore PARP-related cellular responses in different disease contexts.

2 Methods

MSD® MULTI-ARRAY Electrochemiluminescence Technology

- Minimal non-specific background and strong responses to analyte yield high signal-to-background ratios.
- The stimulation mechanism (electricity) is decoupled from response (light signal) minimizing matrix interference.
- Only labels bound near the electrode surface are excited.
- Labels are stable, non-radioactive, and directly conjugated to biomolecules.
- Emission at ~620 nm eliminates problems with color quenching.
- Multiple rounds of label excitation and emission enhance light leve and improve sensitivity.
- Carbon electrode surface has 10X greater binding capacity than polystyrene wells.

Figure 1. Electrochemiluminescence (ECL) technology and MULTI-ARRAY platform from MSD.

MSD R-PLEX Assay Protocol

- Add 25 µL of biotinylated capture antibody to each well of an MSD Streptavidin plate.
- Incubate for 1 hour then and wash 3 times.
- Add 25 µL of diluent then 25 µL of sample or PAR polymer calibrator.
- Incubate for 1 hour then wash 3 times.
- Add 50 µL of SULFO-TAG™ labeled detection antibody.
- Incubate for 1 hour.
- Wash 3 times and add 150 µL of Read Buffer to each well.

Calibration curves were produced from 4-fold dilutions of PAR polymer.

Intracellular PAR Sensitivity

To induce PAR synthesis at two levels, MCF-7 cells were treated with H_2O_2 and lysed, and separately MCF-7 cell lysates were treated directly with H_2O_2. Lysates were serially diluted (2-fold) and the PAR concentration determined using the R-PLEX PAR Assay to assess sensitivity.

Functional PAR Inhibition

PC3, HEK293, and MDA-MB-231 cells were treated with PARP inhibitors for 24 or 72 hours in multi-well culture plates to assess inhibition of PAR levels using the MSD R-PLEX assay. $3x10^5$ cells/well were added to 6-well plates and treated with a high concentration of PARP inhibitor. The percent of PAR inhibition was calculated as a ratio of ECL signal of the drug-treated condition to DMSO control.

IC_{50} values for each inhibitor were calculated from treatment of PC3 cells. $1x10^4$ PC3 cells/well were incubated with PARP inhibitor for 72 hours and lysates collected in 96-well plates. Lysates were run on the R-PLEX PAR Assay. Electrochemiluminescent (ECL) signals were normalized to DMSO control and the data were modeled using Four Parameter Logistic (4PL) curves to generate the IC_{50} of each PARP inhibitor.

Cell Viability

Following PARP inhibitor treatment, viability of cancer cells (PC3, MDA-MB-231) and normal cells (HEK293) was assayed using the CellTiter-Glo assay. $1x10^4$ cells were seeded in 96-well plates and treated with PARP inhibitors for 24 or 72 hours. At assay endpoint, luminescence was determined for all test conditions and normalized to 0.1% DMSO as 100% viability.

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3 Anti-PAR Antibody Screening and Selection

Ten potential anti-PAR antibody pairs were screened using the MSD R-PLEX protocol at two PAR polymer concentrations. Three candidate pairs were selected based on signal:background ratio. Eight point calibrator curves run with the three pairs showed comparable sensitivity, dynamic range, and Hill slope. Antibody Pair 3 was selected for the R-PLEX PAR Assay.

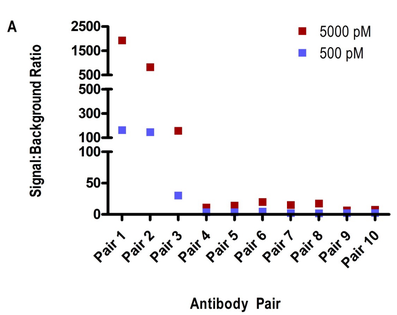

Figure 2. **A**: Antibody screening of anti-PAR antibody pairs. Two concentrations of PAR polymer were used to determine the signal:background ratio.
B: Calibration curves of three candidate antibody pairs to calculate the Hill slope, LLOD and LLOQ.

	Ab Pair 1	Ab Pair 2	R-PLEX Pair
Hill Slope	1.24	0.99	1.01
Estimated LLOD (pM)	1.49	3.2	1.82
Estimated LLOQ (pM)	22.19	10.63	23.5

4 PAR Sensitivity

We serially diluted cell lysates and determined the PAR concentration to assess the sensitivity of the R-PLEX PAR Assay. High and low PAR-expressing samples were detectable at input protein concentrations down to 0.1 µg/well. Approximately 2 logs of separation between high and low PAR-expressing samples were observed with an overall assay dynamic range spanning >3 logs.

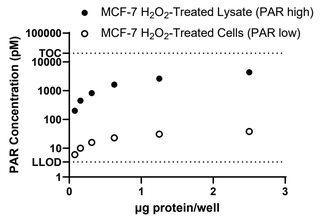

Figure 3. Dilutional linearity of intracellular PAR. Cells (open circles) or collected lysates (closed circles) were treated with H_2O_2 and PAR concentration determined (TOC = top of curve; LLOD = lower limit of detection).

5 Cell Viability with PARP Inhibitors

Concentrations of PARP inhibitors significantly above the reported IC_{50} did not impact cell viability in all cell models tested at 24 hours, and minimally at 72 hours. IC_{50} values could not be determined from cell viability assays.

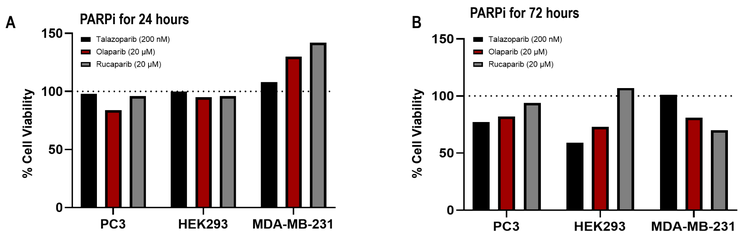

Figure 4. A & B: Viability of cancer cells (PC3, MDA-MB-231) or normal cells (HEK293) using the CellTiter-Glo assay following PARP inhibitor treatment. Data are represented as the luminescence from the test condition (PARP inhibition) to 0.1% DMSO control (dashed line). $1x10^4$ cells were added per well in a 96-well plate.

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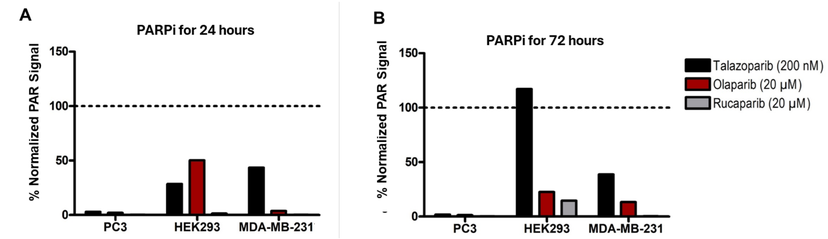
6 Functional Assessment of the R-PLEX PAR Assay Using PARP inhibitors

We conducted functional assessment of the R-PLEX PAR Assay by treating BRCA mutant (PC3), BRCA wild-type (MDA-MB-231), and normal cells (HEK293) with talazoparib, olaparib, and rucaparib and evaluating intracellular PAR levels. ECL signals were normalized to 0.1% DMSO. PAR signal was reduced >95% in the PC3 cell line following PARPi at 24 and 72 hours.

PAR inhibition in HEK293 and MDA-MB-231 varied based on compound incubation time and PARP inhibitor used. Therefore we explored the dose-response in only PC3 to generate complete dose-response curves. IC_{50} values were comparable (±3-fold change) to previously reported results demonstrating the utility of the R-PLEX PAR Assay.



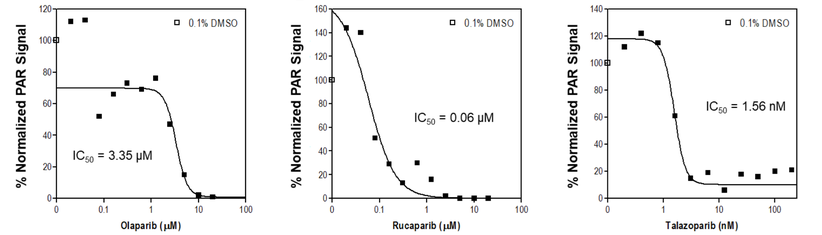


Figure 5. A & B: Normalized PAR signal from lysates of PARPi treated cell lines. $3x10^5$ cells/well in a 6-well culture plate were treated with PARPi for 24 or 72 hours. Lysates were collected and 10 µg of protein was added to the R-PLEX PAR Assay. ECL signals were normalized to control (dashed line).
C-E: PC3 cells were treated with olaparib (C), rucaparib (D), or talazoparib (E) for 72 hours. 5 µg of cell lysate protein was tested in duplicate in the MSD R-PLEX PAR Assay. ECL signal normalized to the control was plotted and IC_{50} values were calculated from the dose-response curve.

7 Conclusions

- Electrochemiluminescence-based detection of PAR is robust, and sensitive, and covers a broad dynamic range.
- The assay uses low quantities of input protein to quantify intracellular PAR.
- The sensitivity of the R-PLEX PAR Assay can detect modulation of the intracellular PAR levels after a 24 hour incubation with inhibitors. Other PAR assays typically require 5-7 days.
- Cell viability assays did not identify the IC_{50} of PARP inhibitors over the time course evaluated.
- The assay detects time- and dose-dependent modulation of PAR levels allowing for triage of biologics or small molecules that impact intracellular PAR.

8 References

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