Laser Microirradiation Assay: Tracking DNA Damage Response (DDR) kinetics

Guido Zagnoli Vieira and Jo Francis

Mechanistic and Structural Biology, Discovery Sciences, R&D, AstraZeneca, Cambridge CB4 0WG, U.K.

Abstract

Laser microirradiation assay (LMA) is the gold standard for the study of kinetics of proteins involved in DNA damage response (DDR). The technique has been widely applied to better understand DNA repair inhibitors mode of action. Currently, olaparib, an inhibitor of the PARP enzymes, has been successful in delivering targeted therapy in homologous recombination deficient tumours. Nonetheless the generation of better or alternative targets are still needed and are widely investigated. Here, we use olaparib to show that we can use a modified protocol of the LMA to assess inhibition of DNA repair in live cells. We believe that this modified workflow will allow for the better characterisation of upcoming inhibitors.

Introduction

- Genome instability is one the main hallmarks of cancer[1]. Poly(ADP-Ribose) Polymerase 1 (PARP1) enzyme is involved in multiple pathways of the DNA damage and response in cells. olaparib[2], a PARP inhibitor works by selectively killing homologous recombination repair defective cells.
- Laser microirradiation has been widely used to measure DDR kinetics[3]. Recruitment kinetics of PARP1 and its downstream partner X-ray repair cross-complementing protein 1 (XRCC1) to laser induced damage has been long observed using this technology[4,5].
- Here, we use the previous observation that olaparib ablates XRCC1 recruitment to laser damage[6], to build a high throughput laser microirradiation assay to aid future drug discovery strategies.

Methods

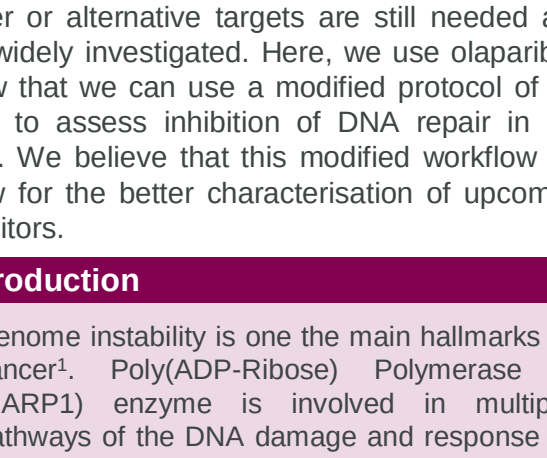


Figure 1 - An overview of the laser microirradiation assay (LMA) – The LMA measures the recruitment of fluorescently labelled proteins to sites of DNA damage. Here, a green fluorescence protein (GFP) tagged XRCC1, is expressed and localized to the nuclei of U-2-OS cell line (1). Prior image acquisition, regions on interest are selected in the nuclei (2). Following incorporation of a sensitization agent, a highly focused laser beam at 405nm introduces localized DNA damage via the production of reactive oxygen species (3). Following DNA damage, the accumulation/recruitment of the protein of interest is measured for a determined time, here 150 seconds (s).

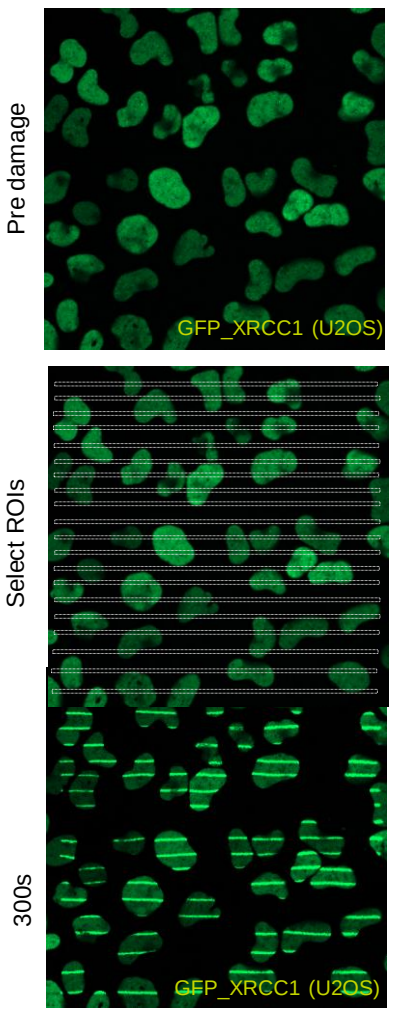


Figure 2 - Increasing throughput of LMA – Using a modified workflow where multiple regions of interest (ROIs) are generated across the field of view (middle image), we can measure the recruitment of our protein of interest in multiple cells simultaneously. This unbiased high throughput LMA (HLMA) approach allows measurement of hundreds of cells for a more robust dataset.

Results

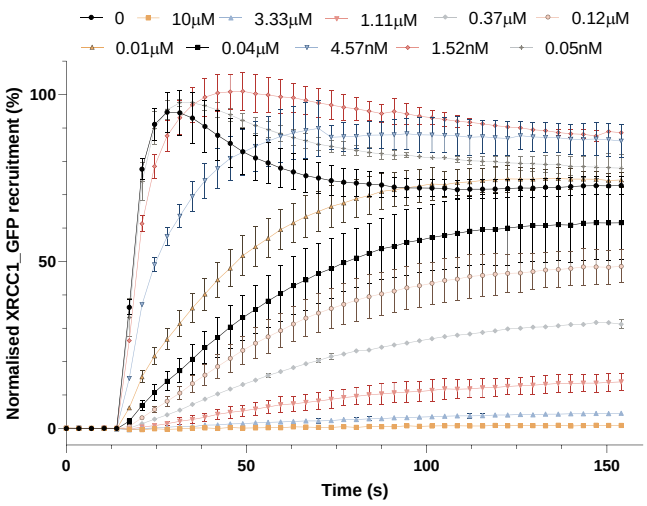
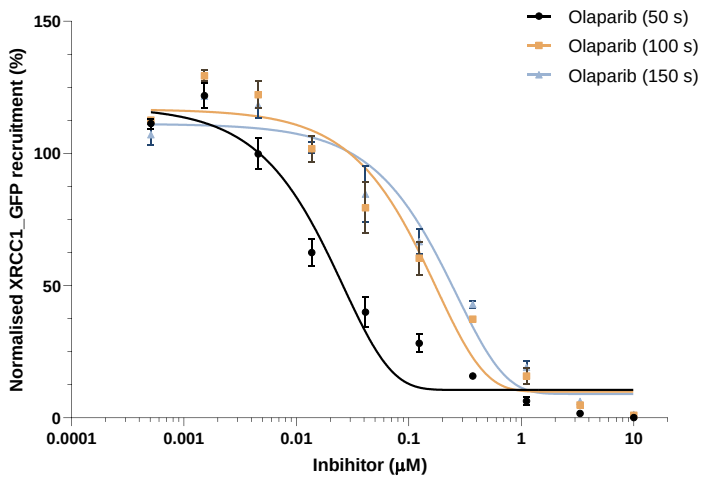


Figure 3 – olaparib dose response using HLMA– XRCC1 recruitment to sites of laser damage is mediated by its high affinity to Poly-ADP-Ribosylation (PARylation) generated by PARP enzymes[4]. Here, we show that treatment with multiple concentrations of olaparib up to two hours prior inducing damage via laser microirradiation, results in a delayed recruitment of XRCC1 to damage sites. XRCC1 recruitment is measured in two field of views with >100 cells per condition. Average recruitment per ROI is normalized to data acquired prior damage and to regions without damage to account for photobleaching effects. Data is from two independent experiments ± SEM.



Time (s)	IC50 (nM)
50	015.57
100	102.89
150	177.65

Figure 4 - Generation of PARylation IC50 using XRCC1 as readout – Using normalized measurement of XRCC1 at three distinct timepoints, 50, 100 and 150 seconds(s), we can draw IC50 values for live cell inhibition of PARP. Data as shown in Fig 3 but normalized to vehicle control to each timepoint.

Conclusions

- Using fluorescently labelled XRCC1, we have successfully setup the conditions to introduce localized DNA damage via laser microirradiation (Fig 1).
- Using the Zeiss Cell Discoverer 7 system, we have been able to increase the throughput of our LMA. This new workflow, increases output by damaging simultaneously multiple cells from a single field of view (Fig 2). Additionally, it simplifies data analysis as the average recruitment of the protein of interest can be derived from the changes in the average signal within a region of interest instead of a single nuclei.
- The modified high throughput LMA has successfully reproduced previously published dynamics for XRCC1 in the presence and absence of the PARP inhibitor olaparib[6] (Fig 3).
- The analysis of the dose response kinetics of XRCC1 in the presence of olaparib at different intervals allows for a better understating of inhibitor effect over time and a better understating of PARylation dynamics in cells (Fig 4). Interestingly, the IC50 values obtained correlates with efficacy values previously measured for BRCA defective MDA-MB-436 cells with IC50 of 200nM[2].

References

1. Hanahan, Douglas, ***Cancer Discovery***, 2022
2. Menear K. *et al.*, ***Journal of Med. Chemistry***, 2008
3. Rogakou E. *et al.*, ***Journal Cell of Biology***,1999
4. Mortusewicz O. *et al.*, ***Nucleic Acid Research***, 2007
5. Mahadevan J *et al.*, ***DNA Repair***, 2019
6. Campalans A. et al., ***Nucleic Acid Research***, 2013

Acknowledgements

Josep Forment and DDR Team at AstraZeneca for discussion and support.

Supported by AstraZeneca

Presented at the ELRIG Drug Discovery, London, 03 October 2022