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Novel functional dSTRIDE-HR assays to report on the status of homologous recombination repair in cancer cells

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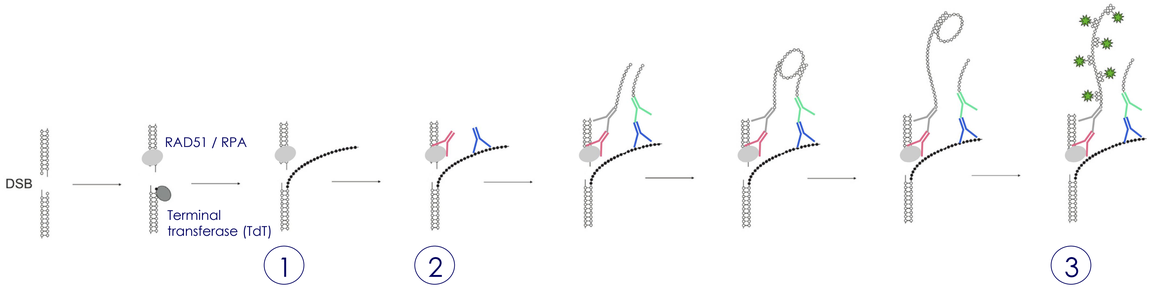
INTRODUCTION

Determination of the HR status of a cell is of great importance for the use of already approved and development of new DDR inhibitors. While classic approaches such as immunofluorescence-based detection of RAD51 or genomic HRD assays provided a wealth of information, functional biomarkers are still missing. We report here the development, optimization and validation of two complementary, HR-specific functional assays. The assays are based on STRIDE platform technology and enable the direct detection of double-strand DNA breaks that are accompanied by either RPA (dSTRIDE-RPA) or RAD51 (dSTRIDE-RAD51) proteins. The neccessary coincidence of a DSB and a HR factor assures that the signals occur only at sites of HR repair and allows to avoid detection of accumulation of these proteins at sites that are not directly related to the DDR response.

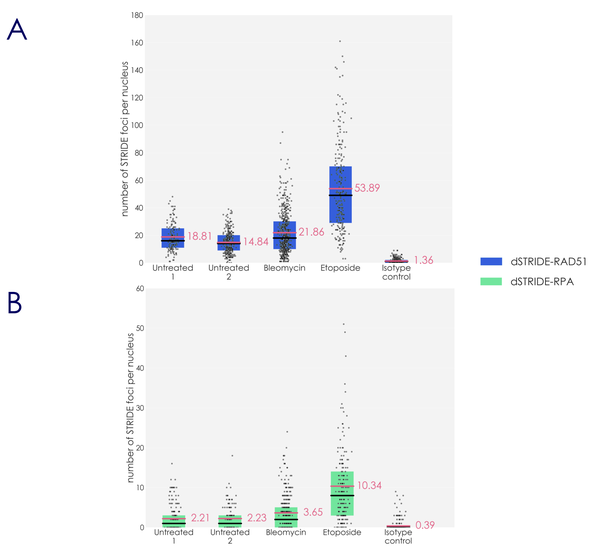
Figure 1. Schematic representation of the dSTRIDE-HR assays.



dSTRIDE-HR assays allow direct detection of a DNA break and HR-related proteins. The main steps in the procedure are 1) enzymatic modification of a free DNA end, 2) antibody-based detection of modified nucleotides and the protein of interest and 3) signal amplification using RCA reaction.

Figure 2. The level of RPA or RAD51 related DSBs in U2OS cells .

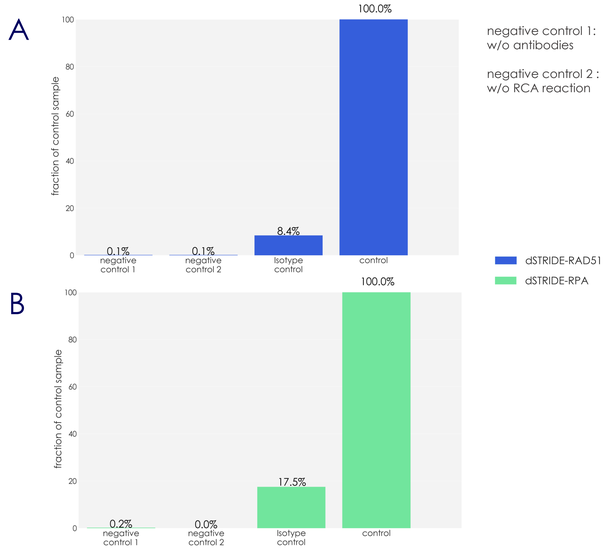
Double-strand DNA breaks were induced in U2OS cells using bleomycin or etoposide.



Results of 3D quantitative image analysis – comparison of A) dSTRIDE-RAD51 and B) dSTRIDE-RPA readouts in U2OS cells. Each dot represents a single cell, the mean value is depicted as a red horizontal line and median as black.

Figure 3. Assay validation: negative controls in U2OS cells.

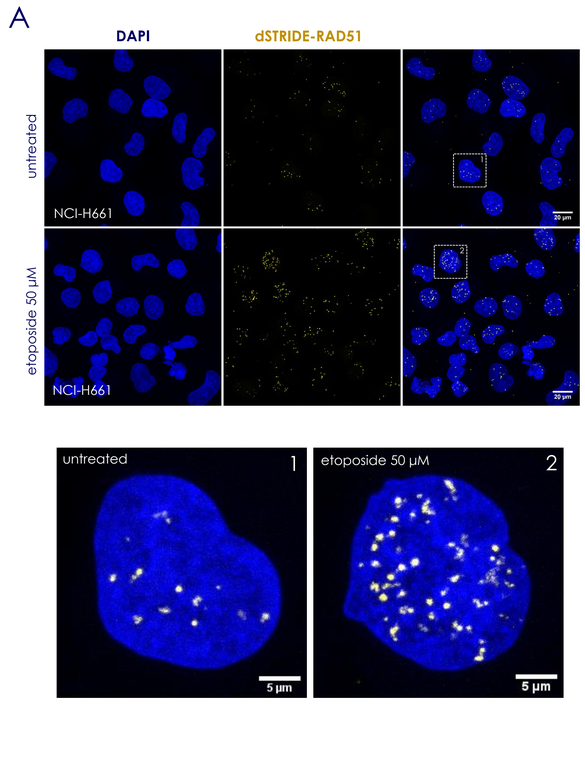
Technical negative controls were tested in dSTRIDE-HR assays.



Results of 3D quantitative image analysis – comparison of negative controls in A) dSTRIDE-RAD51 and B) dSTRIDE-RPA assays. Top of each bar represents the fraction of signal in relation to the full procedure.

Figure 4. Comparison of dSTRIDE, dSTRIDE-RPA and dSTRIDE-RAD51 readouts in cell models with different HR-status.

Double-strand DNA breaks were induced in two non-small lung cancer cell lines using etoposide. In both cell lines treatment with etoposide resulted in a dose-dependent increase in the number of DSBs and RPA-associated DSBs. An increase in the number of RAD51-associated DSBs was detectable only in NCI-H661 cells, which highlights the difference in HR-proficiency between the cell models.



A) Representative images showing non-small lung cancer NCI-H661 cells in which HR-related DSBs were labelled using the dSTRIDE-RAD51 assay. An increase in the number of foci is visible is cells treated with 50 µM etoposide.

B) Results of 3D quantitative image analysis – comparison of readouts obtained using three separate assays. No increase in the number of RAD51-related DSBs is detected in NCI-H1693 cells. Top of each bar represents the mean.

CONCLUSIONS

Assay validation and the presented use-case confirm that the dSTRIDE-HR assays can be succesfully applied to inform about HR status of a cell. High signal to noise ratio, very low level of false positive readouts and the direct detection of a DSB make the assays an attractive alternative to often misleading classic IF detection of RAD51. Further studies are needed to assess whether the assays can be used to discriminate cells or tumours with different genetic backgrounds at steady state, i.e. without stimulation with exogenous agents.