sSTRIDE as a technology platform for the detection and measurement of DNA breaks in various DNA repair pathways

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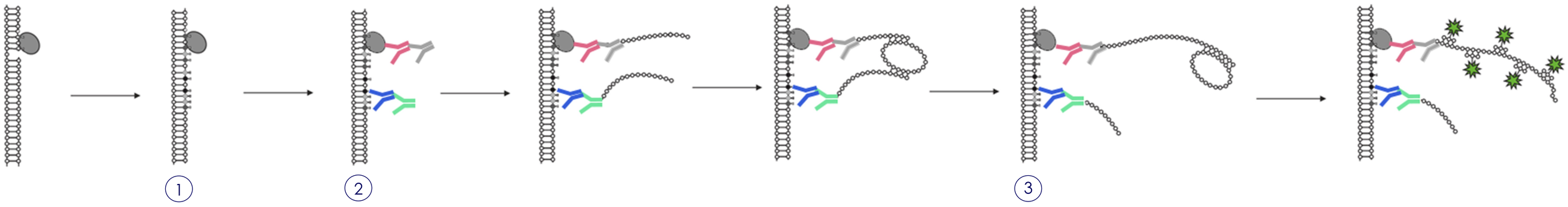
INTRODUCTION

sSTRIDE allows direct detection and precise quantification of single-strand DNA nicks and gaps. However, the assay can be customized and adjusted for detection of DNA breaks repaired by a specific DNA Damage Response pathway. Here we present two examples of assay variants: sSTRIDE-PMS2 and sSTRIDE-SMUG1. We discuss how those variants were developed, validated and how their application can support the development of DDR targeting therapeutics.

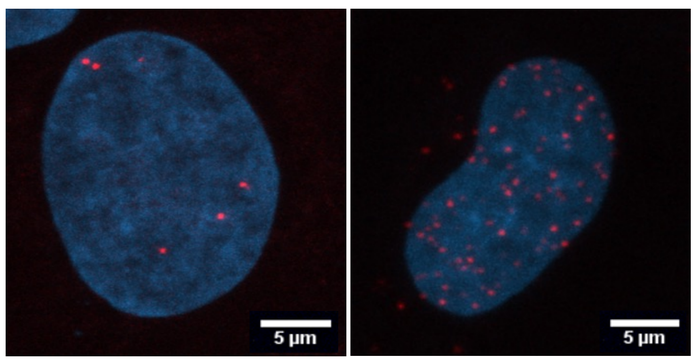
Figure 1. Schematic representation of the sSTRIDE-based customized assay variants.



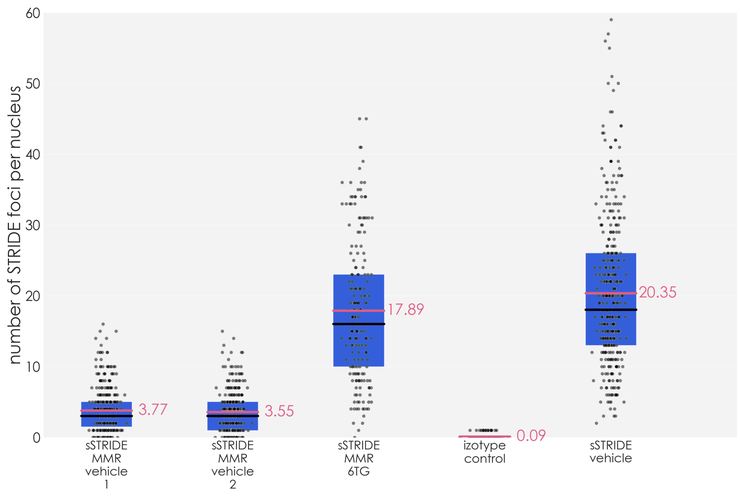
sSTRIDE assay allows direct detection of single-strand DNA nicks and gaps. The main steps in the procedure consist of:

1) enzymatic incorporation of modified nucleotides, 2) antibody-based detection of modified nucleotides and (in customized assay variants) a DDR-specific protein; and 3) signal amplification using RCA reaction.

Figure 2. sSTRIDE-PMS2: detection of MMR-specific SSBs (single-strand DNA breaks) in U2OS cells.

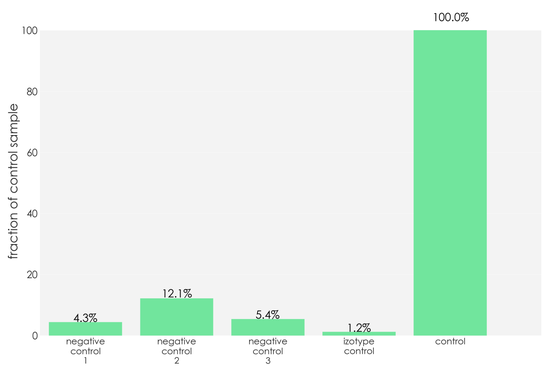


Nuclei with MMR-specific SSBs (left) and all types of SSBs.



A comparison of MMR-specific SSBs in vehicle and 6TG treated sample, as well as traditional sSTRIDE and isotype control, based on a 3D quantitative image analysis. Each dot represents counts from a single nucleus. The mean value is depicted as a red horizontal line, and median as a black one.

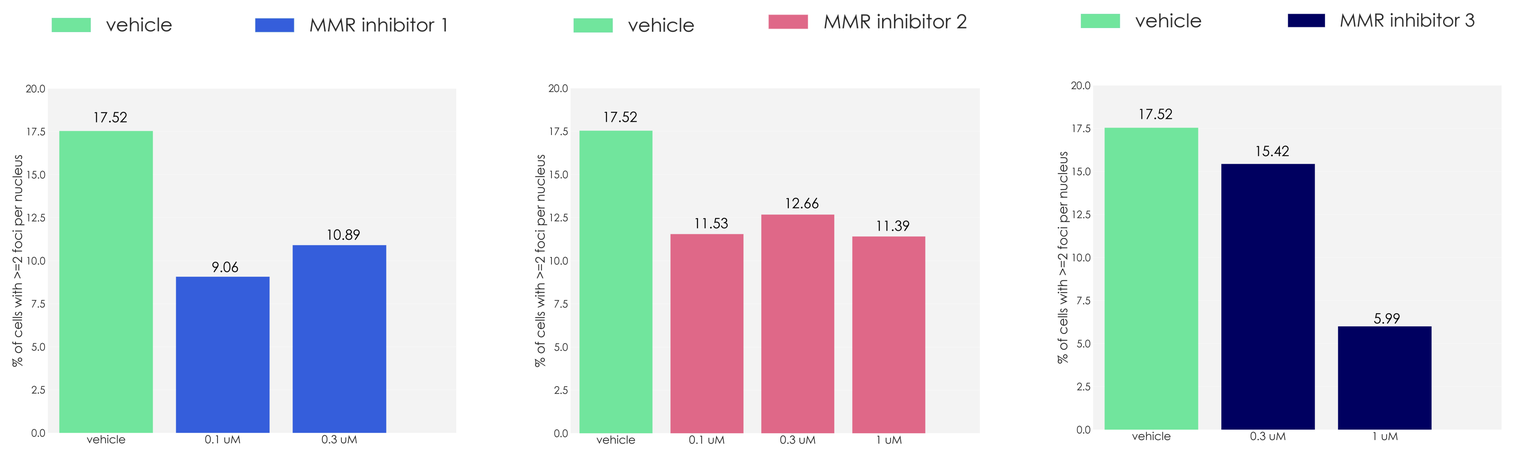
Figure 3. sSTRIDE-PMS2: assay validation - negative controls in U2OS cells.



All negative controls show an order of magnitude lower unspecific signal when compared with control in sSTRIDE-MMR assay.

Figure 4. sSTRIDE-PMS2: application of the assay test the effect of MMR inhibitors.

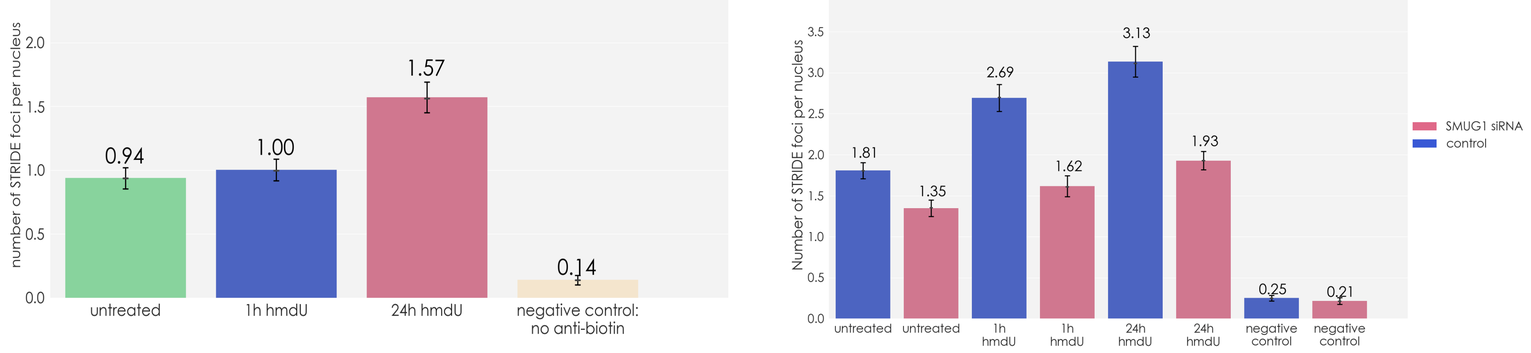
Level of MMR-specific SSBs in HAP1 cells before (vehicle) and after treatment with MMR inhibitor 1 (left), MMR inhibitor 2 (center), and MMR inhibitor 3 (right). Quantitative results were obtained based on a 3D image analysis, where each dot represents counts from a single nucleus. Mean and median are marked with red and black horizontal lines, respectively.



Percentage of cells with two or higher MMR-specific SSBs per nucleus before (vehicle) and after treatment with MMR inhibitors. 2 SSBs per nucleus is the 90th percentile threshold established based on MMR-specific counts in vehicle sample. Quantitative results were obtained based on a 3D image analysis.

Figure 5. sSTRIDE-SMUG1: detection of SSBs formed upon hmdU treatment.

sSTRIDE-SMUG1 is another assay variant that was developed based on the sSTRIDE technology. The assay detects the spatial coincidence of a single-strand DNA break and SMUG1 protein, a uracil-DNA glycosylase which is a part of the Base Excision Repair (BER) pathway.



Incubation of HAP1 cells with 5-hydroxymethyl-deoxyuridine (hmdU) for 24 hours resulted in an increase in the numer of detected sSTRIDE-SMUG1 foci, confirming that SMUG1 is contributing to a repair pathway aimed at removing this cytotoxic nucleoside from the genomic DNA.

The new assay was validated by knocking-down SMUG1 in HAP1 cells using siRNA. SMUG1 expression silencing resulted in a decrease in the number of sSTRIDE-SMUG1 foci in all tested conditions.

CONCLUSIONS

Assays measuring the level of SSBs in the context of specific DNA repair proteins, such as sSTRIDE-PMS2 and sSTRIDE-SMUG1 are important tools enabling direct insight into the biology of different repair pathways, as well as activation of these pathways as a consequence of e.g., drug treatment. The ability to develop and validate new customized assay variants based on STRIDE platform technology opens a new avenue in research focused on harnessing the DDR for therapeutic purposes.