STRIDE™: a platform for quantitative DNA damage response dissection

intoDNA S.A., Krakow, Poland

M. Białecka, O. Wójcikowska, K. Białas, D. Jaśniak, J. Lechowski, M. Bartyńska, K. Stepien, F. Sierpowski, K. Schirmeisen, Z. Prucsi, A. Waligórska, M. Kordon-Kiszala, K. Solarczyk

— magda@intodna.com
— kamil@intodna.com
— www.intodna.com

Introduction STRIDE™ directly labels the sites of DNA breaks, making it the most proximal DNA damage marker available to date. Proprietary STRIDE™ technology is a fluorescence-based method that directly labels DNA damage sites and detects individual DNA lesions. Its strong signal amplification enables robust and reliable quantification of DNA damage. Combination of classic and functional assays provides detailed information on the degree of involvement of various DNA repair mechanisms.

Common core for all assays

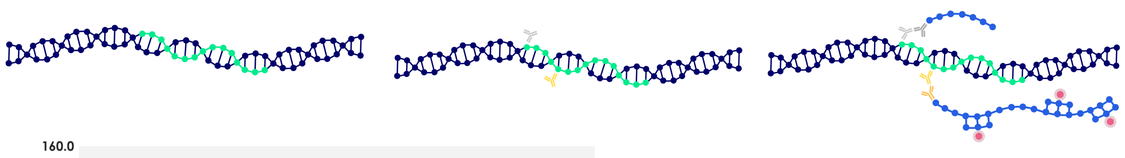
LABELING. Direct enzymatic incorporation of nucleotide analogues ■ to DNA ends within the breaks.

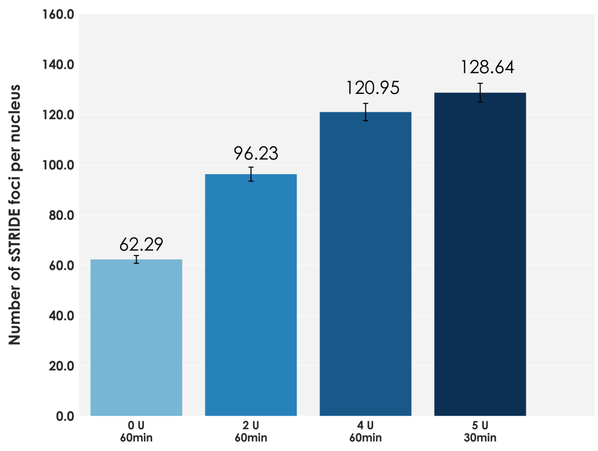
RECOGNITION. Attachment of a pair of primary antibodies ■ ■ to the labelled DNA ends or a protein of interest

DETECTION. Secondary antibodies ■ ■ binding and strong STRIDE™ signal ■ enhancement on closely localized antibody complexes.

Classic STRIDE™ assays

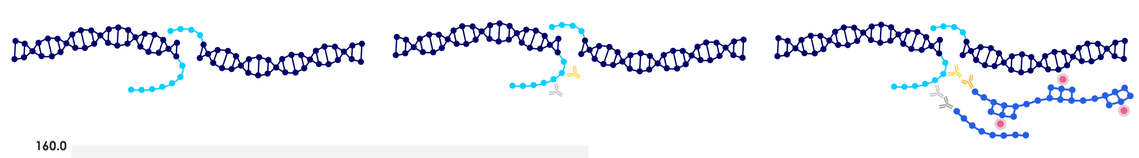
sSTRIDE

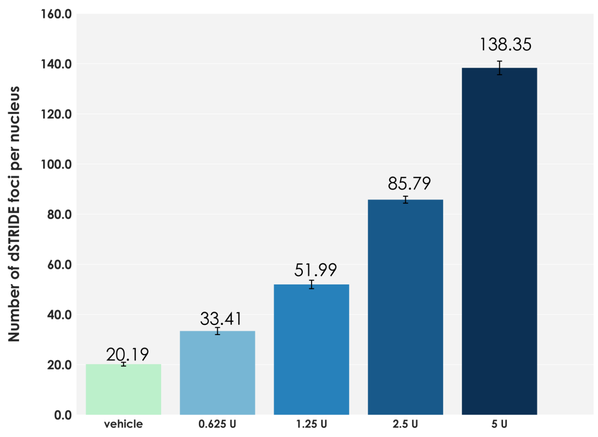




Nb.BtsI is a nicking endonuclease that cleaves only one strand of DNA on a double- stranded DNA substrate. There is a dose-dependent increase in the number of sSTRIDE foci with increasing concentration of the enzyme.

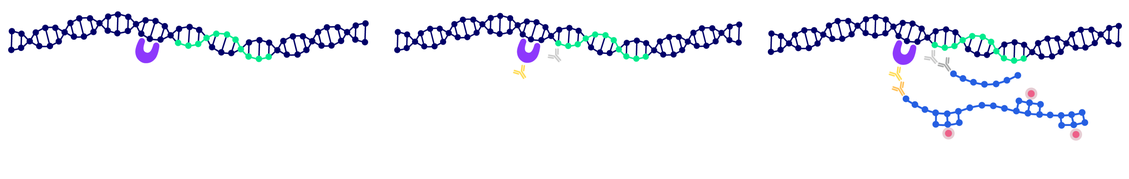
dSTRIDE

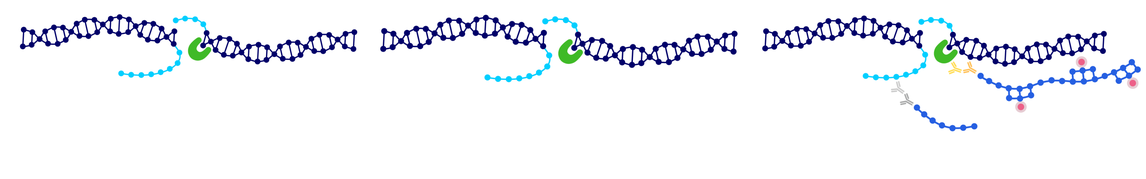




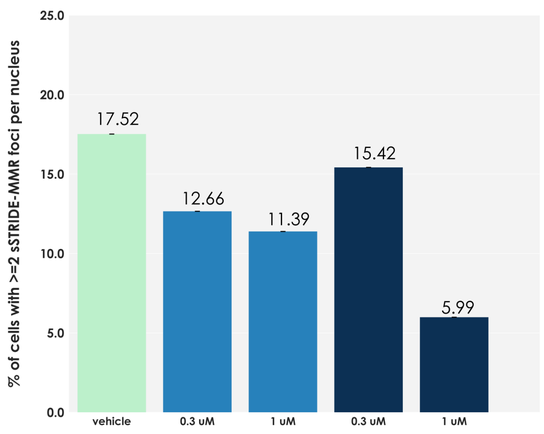
FokI restriction enzyme recognizes GGATG(9/13)^ site. There is a dose-dependent increase in the number of dSTRIDE foci with increasing concentration of the enzyme.

DNA Damage Response Functional STRIDE™ assays



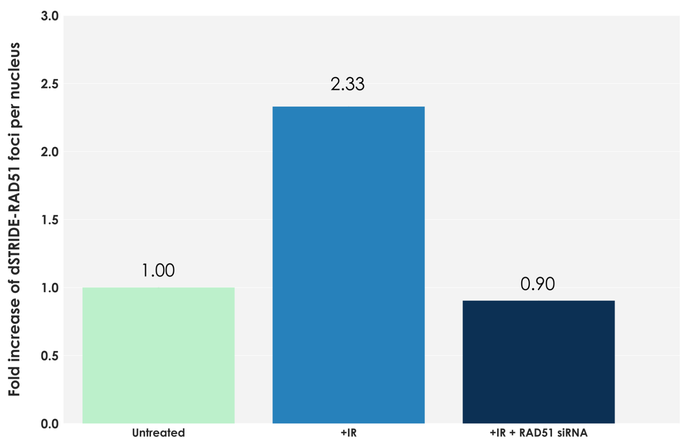


sSTRIDE-PMS2. PMS2 is a key component of the mismatch repair system that functions to correct DNA mismatches and small insertions and deletions



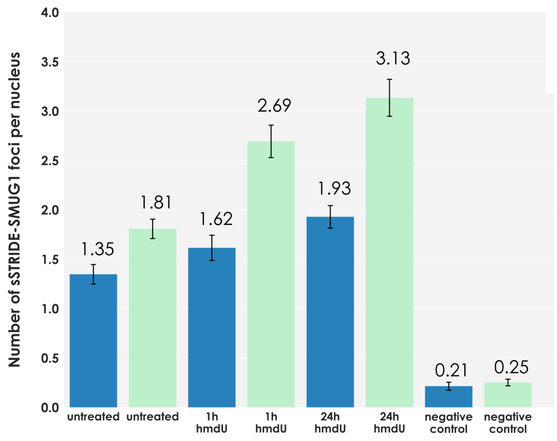
Percentage of cells with two or more MMR-specific SSBs per nucleus before (vehicle) and after treatment with MMR inhibitors. 2 SSBs per nucleus is the 90th percentile threshold established based on MMR-specific counts in vehicle sample. Quantitative results were obtained based on a 3D image analysis.

dSTRIDE-RAD51. RAD51 is an enzyme which assists in repair of DNA double strand breaks



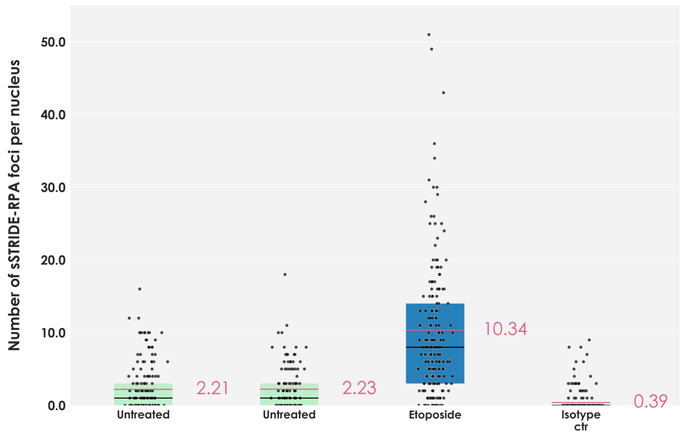
Ionizing radiation (IR) induced RAD51-specific double-strand DNA breaks when compared to untreated cells. Silencing of RAD51 (RAD51 siRNA) abolishes RAD51 recruitment to IR-induced DSBs, bringing it to the level comparable with the untreated sample. All results were normalized to the number of dSTRIDE-RAD51 foci in the untreated sample.

sSTRIDE-SMUG1. SMUG1 is a uracil-DNA glycosylase which is a part of the Base Excision Repair (BER) pathway



Incubation with 5-hydroxymethyl-deoxy-uridine (hmdU) resulted in an increase in the number of sSTRIDE-SMUG1 foci, confirming that SMUG1 is contributing to a repair pathway aimed at removing this cytotoxic nucleoside from the genomic DNA. Knocking-down SMUG1 using siRNA resulted in a decrease in the number of sSTRIDE-SMUG1 foci compared to the control.

dSTRIDE-RPA70. RPA70, as a part of the replication protein A, plays an essential role in DNA replication, recombination and repair

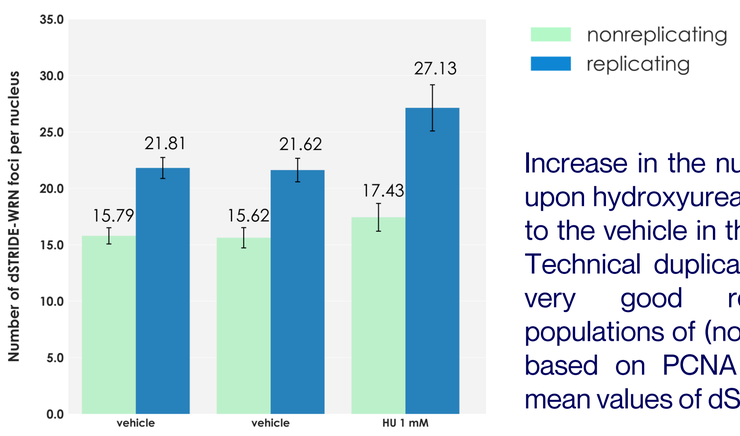


Double-strand DNA breaks were induced using etoposide. Each dot represents count from a single nucleus, the mean value is depicted as a red horizontal line and median as black. Isotype control samples show a very low background level of signals.

Conclusions

STRIDE™ technology provides a highly sensitive method for detecting even subtle changes in the levels of DNA breaks. Its versatility allows for the analysis of both single- and double-strand DNA breaks across various cellular compartments. Importantly, STRIDE™ offers specificity by directly measuring DNA breaks without interference from other cellular events and operates independently of DDR pathways. Assays measuring the level of DNA breaks in the context of specific DNA repair proteins report about the biology of different repair pathways and potentially, about perturbance of these pathways as a consequence of 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 DNA damage response (DDR) for therapeutic purposes and positions STRIDE™ as a crucial asset for advancing our knowledge of DNA damage mechanisms and therapeutic responses.

dSTRIDE-WRN. WRN is ATP-dependent helicase important in the maintenance of genome stability, DNA repair, replication, transcription and telomere maintenance



Increase in the number of WRN-associated DSBs upon hydroxyurea (HU) treatment when compared to the vehicle in the population of replicating cells. Technical duplicates of untreated samples show very good reproducibility. Representative populations of (non)replicating cells were selected based on PCNA co-staining. Bar plot showing mean values of dSTRIDE-WRN foci per nucleus.