STRIDE®platform: direct DNA break detection and functional repair profiling

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intoDNA
where DNA breaks, our insight begins

Introduction

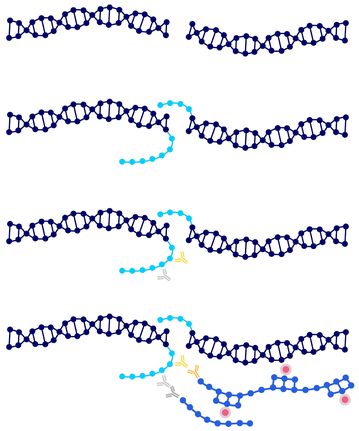
Many cancer therapies act by creating DNA strand breaks, yet breaks are usually measured indirectly, through DDR markers whose signal depends on the cell's repair machinery. STRIDE is an image-based platform that directly labels individual single-strand (sSTRIDE) and double-strand (dSTRIDE) DNA breaks *in situ*,at single-cell resolution. By coupling direct break detection with proximity-based recognition of a repair protein, we developed STRIDE Functional DDR assays that show which factor is engaged at the break: RAD51 (dSTRIDE-HR), trapped PARP1 (sSTRIDE-PARP1) or the replication stress proteins pRPA32, pRAD17 and RAD9. Together, they profile DNA damage and the repair response, supporting biomarker discovery and drug development across DDR, ADC and RLT programs.

Technology and Methods

STRIDE® methodology & analysis

- **Direct detection:** High-sensitivity *in situ* labeling for the detection and visualization of DNA strand breaks
- **Single-cell resolution:** Quantitative functional profiling at the individual cell level
- **Sample versatility:** Validated across coverslips, 96-well plates, and clinical tissue (FFPE & fresh frozen).
- **High-dimensional multiplexing:** Seamless integration with cell cycle (Geminin, Ki67, PCNA) and DDR markers.
- **AI-driven quantification:** Automated analysis via CABLES, a custom AI solution for robust, high-throughput image scoring

Model of how dSTRIDE works - direct detection of DSBs



Labelling
Enzymatic modification of a single-strand break with modified ■ nucleotides.

Recognition
Attachment of a pair of primary ■ ■ antibodies to the incorporated nucleotides.

Detection
Secondary ■ ■ antibodies binding and strong STRIDE® signal ■ enhancement on closely localized antibody complexes.

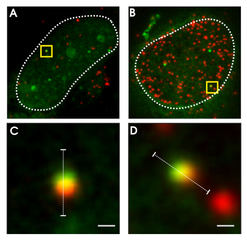
Image acquisition & analysis

Cells were fixed, processed with the STRIDE protocol and counterstained with DAPI. 3D confocal stacks were analysed with CABLES: nuclear masks were built from the DAPI signal and STRIDE foci counted in 3D within each nucleus.

STRIDE Functional DDR assays follows the same principle as the model above, with DSB ends or SSBs labelled and the protein (RAD51, PARP1, RPA) as the partner.

Core assays: dSTRIDE & sSTRIDE

STRIDE detects individual DNA nicks and cuts induced by CRISPR/Cas9

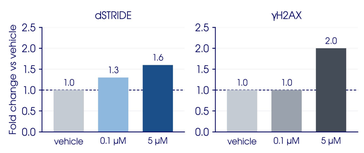


STRIDE sensitivity was demonstrated using a CRISPR/Cas9 system that induces a controlled number of DNA breaks or nicks in U2OS cells.

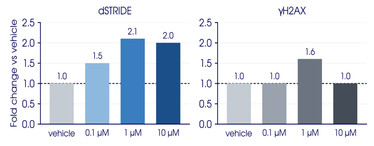
A combination of guide RNAs and fluorescently tagged SpCas9 labels a repetitive sequence on the long arm of chromosome 3 and induces DNA cuts within this region.

Individual DNA breaks (A) and nicks (B) were detected using dSTRIDE and sSTRIDE, respectively. The zoomed in images confirm colocalization of dSTRIDE (C) and sSTRIDE (D) foci with the sites where the cuts or nicks were introduced.

dSTRIDE superior sensitivity enables the detection of subtle changes in DNA damage caused by a DDR inhibitor

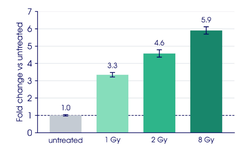


HCT116 + olaparib. dSTRIDE detects DNA breaks already at 0.1 μM olaparib, where γH2AX shows no response. This reflects the lower sensitivity of γH2AX and the delayed engagement of the repair machinery at low drug doses. Fold change vs vehicle, n=3.



DLD-1 + ATR inhibitor (VE-821). dSTRIDE foci increase with ATRi-induced DSBs (up to 2.1-fold), while γH2AX shows mild or no response. A low γH2AX signal could misleadingly suggest less DNA damage, when it may instead reflect disrupted repair signalling. Fold change vs vehicle, n=3.

sSTRIDE detects SSBs induced by IR in human PBMCs

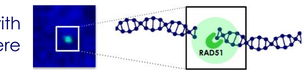


Human PBMCs were irradiated *ex vivo* with 1, 2 or 8 Gy and fixed immediately after IR.

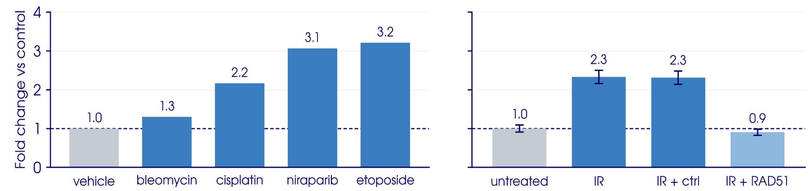
sSTRIDE foci increased with dose, from **3.3-fold at 1 Gy to 5.9-fold at 8 Gy** showing that sSTRIDE quantifies radiation-induced SSBs across a wide dose range.

Functional STRIDE DDR assays

STRIDE Functional DDR assays pair direct break detection with antibody recognition of a repair protein: a signal forms only where the break and the protein are found together.



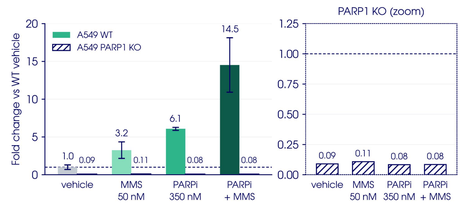
dSTRIDE-HR reports RAD51 engagement at DSBs



Left: U2OS cells treated with bleomycin, cisplatin, niraparib or etoposide; dSTRIDE-HR foci rose with every agent, up to 3.2-fold. **Right:** 6 Gy IR (4 h) raised RAD51-associated DSBs in S-phase HeLa cells 2.3-fold; **RAD51 siRNA leads to signal decrease to the baseline lavel**, while control siRNA has no effect.

Because a DSB and RAD51 must both be present, the assay ignores RAD51 accumulation unrelated to repair.

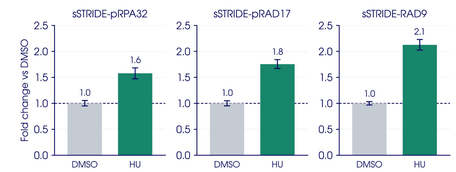
sSTRIDE-PARP1 measures PARP1 trapping at SSBs



A549 WT and PARP1 KO cells treated with MMS (50 nM), a PARPi inhibitor or both.

In WT cells, MMS and PARPi each raised the signal (3.2× and 6.1×), and **the combination gave a 14.5× increase**, consistent with PARP1 trapped at MMS-induced SSBs. In PARP1 KO cells the signal stayed at ~0.1× of WT vehicle in every condition, confirming the readout depends on PARP1.

sSTRIDE detects replication stress factors at SSBs



DLD-1 cellls treated with hydroxyurea ([HU, 2 mM, 6 h]).

HU increased the number of SSBs associated with pRPA32 (1.6×), pRAD17 (1.8×) and RAD9 (2.1×), showing **recruitment of replication stress response proteins to sites of SSBs**.

Conclusions

STRIDE detects individual DNA breaks directly and independently of the DDR machinery, picking up subtle drug-induced damage missed by γH2AX. STRIDE Functional DDR assays add a second layer, showing what happens at the break: RAD51 engagement (dSTRIDE-HR), PARP1 trapping by PARP inhibitors (sSTRIDE-PARP1) and recruitment of replication stress factors, with specificity confirmed by RAD51 knockdown and PARP1 knockout. Quantified per cell on a platform compatible with FFPE tissue, they form one toolbox for mechanism-of-action (MoA) studies, pharmacodynamic (PD) assessment and functional biomarker development.

REFERENCES

1. **Kordon, M. M., Zarębski, M., Solarczyk, K., Ma, H., Pederson, T. & Dobrucki, J. W.** STRIDE—a fluorescence method for direct, specific *in situ* detection of individual single- or double-strand DNA breaks in fixed cells. *Nucleic Acids Res.* **48**, e14 (2020).
2. **Kordon MM, Szczurek A, Berniak K, Szelest O, Solarczyk K, Tworzydło M, Wachsmann-Hogiu S, Vaahtokari A, Cremer C, Pederson T, Dobrucki JW.** PML-like subnuclear bodies, containing XRCC1, juxtaposed to DNA replication-based single-strand breaks. *Nucleic Acids Res.* **49**, 11930–11945 (2021).
3. **Solarczyk, K. & Kordon-Kiszala, M.** Let's not take DNA breaks for granted: the importance of direct detection of DNA breaks for the successful development of DDR inhibitors. *Front. Cell Dev. Biol.* **11**, 1118716 (2023).