Self-DNA Sensor, STING, May Affect Response To Perturbed Replication In Human Cells

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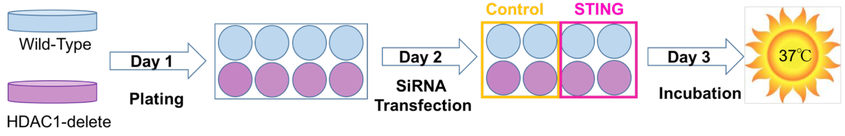
BACKGROUND

- Disruptions in cellular response to perturbed replication compromise genome stability which can lead to inflammation and cancer.
- We conducted a screen for genes that, when downregulated, affect the integrity of newly synthesized DNA during S phase arrest in normal and Histone deacetylase 1 (HDAC1)-deleted cultured human fibroblasts.
- We identified STING, a key activator of innate immune response to foreign DNA as one of such genes.
- STING also activates in response to inappropriate presence of fragmented genomic DNA in the cytoplasm, which is known to be triggered by replication disruptions.

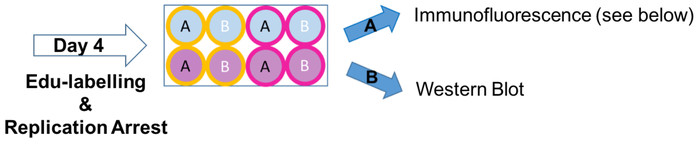
The Goal Of This Study: Confirm and extend our screen result by measuring stability of newly-replicated (i.e. nascent) DNA under conditions of replication disruption in cells with normal and lowered STING expression.

METHODS

Part 1: Establish genotypes



Part 2: DNA-labelling, Inducing replication arrest, and WB

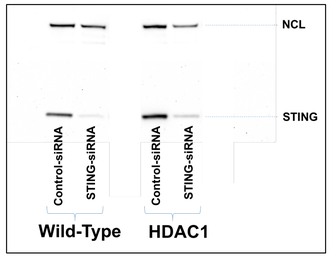


Part 3: Immunofluorescence

- Edu: a thymidine analog that's incorporated into DNA during replication and is used here to measure nascent DNA.
- HU: Hydroxyurea was used to induce S-phase replication arrest.
- WB: Western Blot (to measure STING depletion levels)
- IF: Immunofluorescence

RESULTS

1. Western Blot demonstrates siRNA-mediated depletion of STING protein

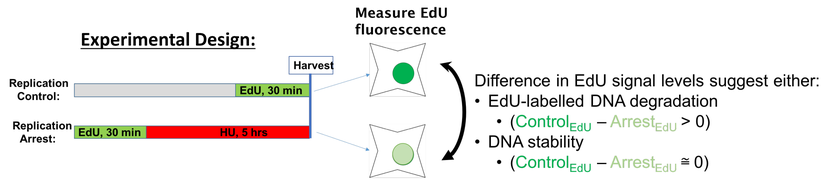


- We can use WB to quantify depletion level.
- Depletion level may vary between experiments and cell lines.

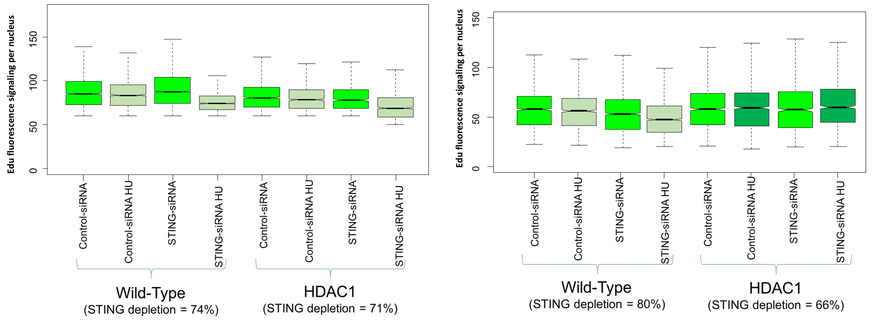
*NCL = nucleolin (internal control)

2. Nascent DNA degradation depends on STING depletion

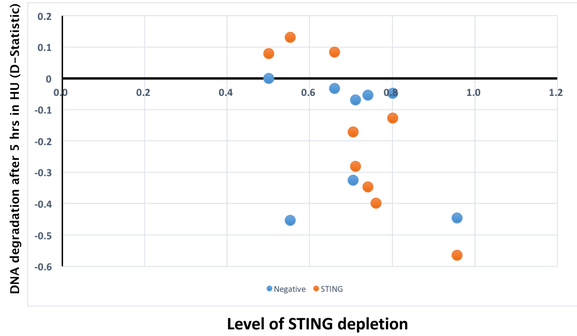
Experimental Design:



2A. Quantitative comparison of EdU fluorescence values in replication-arrested versus non-arrested cells suggests that STING depletion exacerbates loss of labelled (nascent DNA):

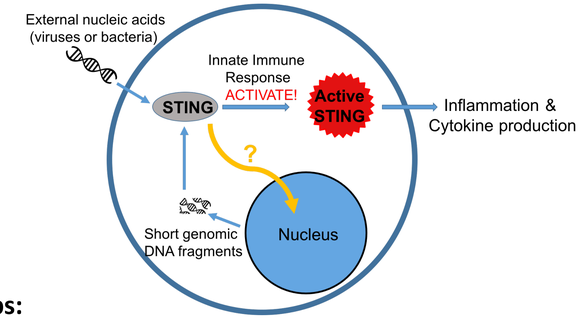


2B. We quantified the difference between EdU signal distributions in arrested vs. non-arrested cells (D statistic) in 4 independent experiments. Here, negative D-values indicate lower EdU signal in arrested cells compared to non-arrested. Plotting D statistic versus STING depletion level suggests that when 70% or more of STING is depleted, EdU signal loss is enhanced.



DISCUSSION

- Degradation of nascent DNA upon replication arrest has been shown to induce STING, but so far it has not been shown that STING activity itself can feedback onto nascent DNA stability. This finding, if confirmed, will provide novel insight into the mechanisms that connect decreased DNA degradation seen in chemoradiation-resistant cancers and STING pathway downregulation.



Next Steps:

- Extend experiments to cancer cell lines that have mutated or downregulated STING (e.g. osteosarcoma U2OS line).
- Use Sidorova lab's DNA fiber assay to measure integrity of nascent DNA at individual replication forks in order to confirm STING's effect.

CONCLUSIONS

- There is more DNA degradation with increased levels of STING depletion.
- Our results suggest that inactivation of innate immune signaling can affect the stability of nascent DNA in the genome under conditions of replication disruptions.
- Relationship between STING and HDAC1 is still unclear although there is some literature that suggests HDAC1 contributes to the induction of the innate immune response.

ACKNOWLEDGEMENTS

Thank you to the Sidorova lab for the mentorship and guidance.