DNA Damage Signalling

INSTITUTE OF CANCER RESEARCH
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Interplay between cellular metabolism and the DNA damage response

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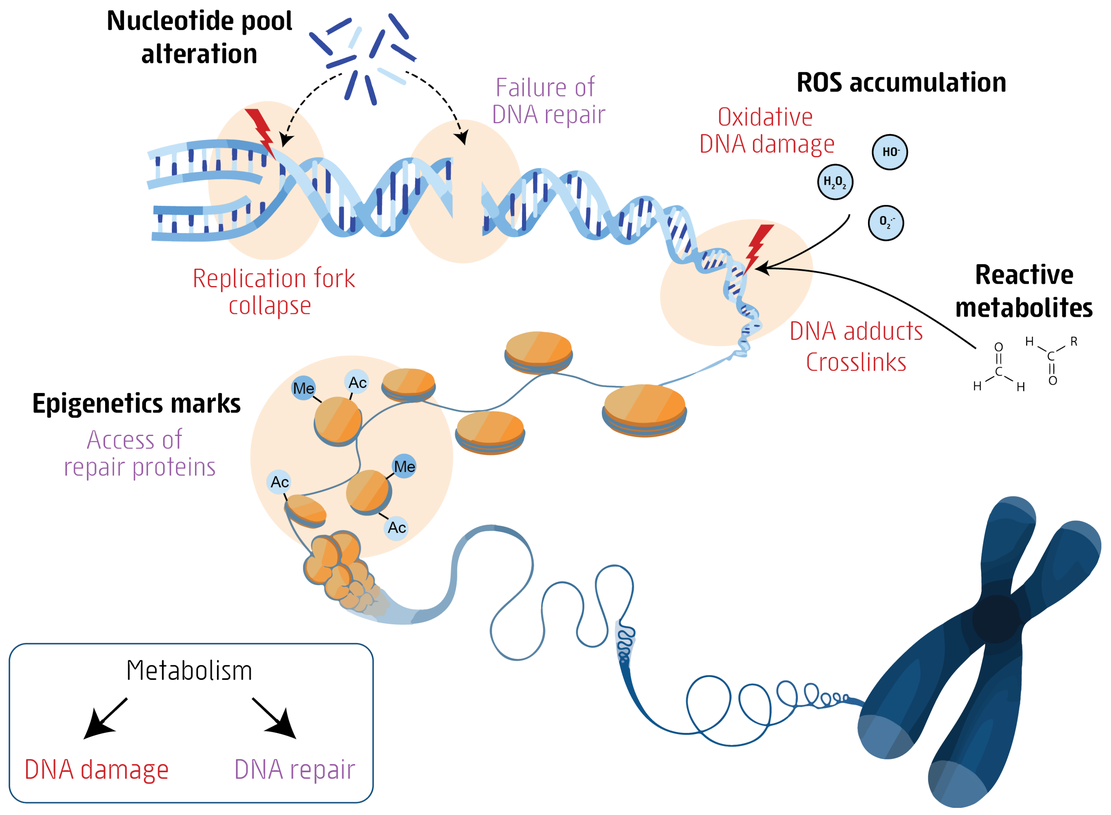
Background and objectives

Compelling evidence has underscored the importance of cellular metabolism in DNA damage and repair[1,2], for example:

- Metabolic reactions generate toxic by-products such as aldehydes, alkylating agents and reactive oxygen species, which can generate crosslinks, DNA adducts and oxidative DNA damage.
- The availability of nutrients impact on the nucleotide pool, which can lead to stalled replication fork and double-strand breaks. Regulation of nucleotide pool is also crucial for DNA repair.
- Chromatin remodeling and epigenetic marks play a crucial role in DNA repair. Some dealkylases use α-ketoglutaric acid, produced from glutamine, as a key substrate and are inhibited by other metabolites such as 2-hydroxyglutarate, fumarate, or succinate, which prevents histone demethylation and subsequent recruitment of homologous recombination factors.
- Cancer cells show both an accumulation of DNA damage and an alteration of DNA repair coupled with modifications in cellular metabolism.

However, a systematic analysis of such interactions has not been yet reported.

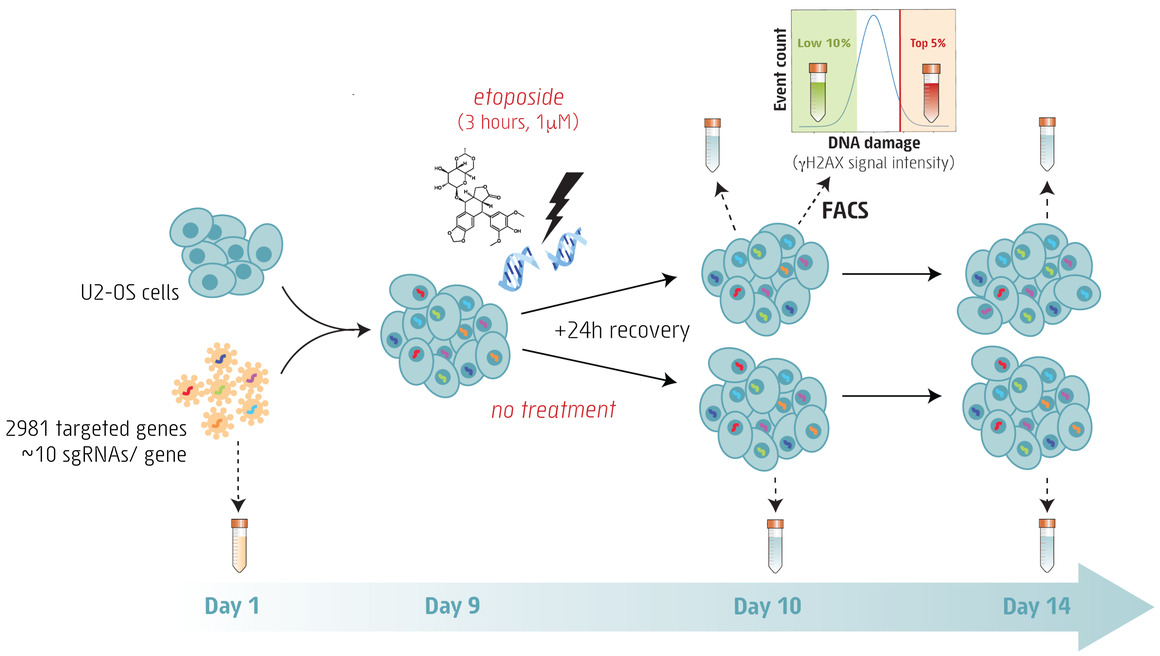
➢ **The overall goal of this project is to functionally explore genetic interactions between metabolism and DNA repair.**



Methods

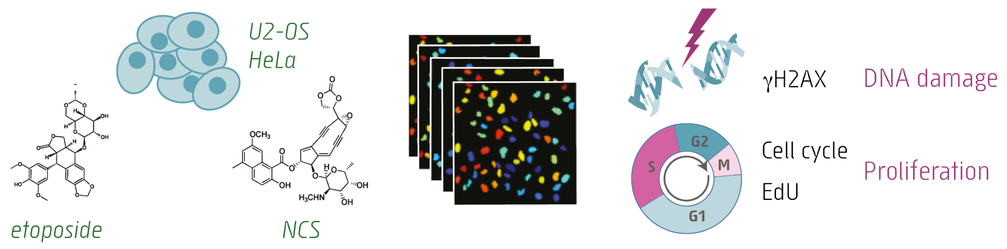
Primary screen: *pooled CRISPR screen with FACS readout*

- Human metabolic CRISPR knockout pooled library from Sabatini lab[3]
- Etoposide treatment: Inhibitor of topoisomerase 2 – induction of DSBs
- Readout: FACS after staining for γH2AX as a proxy of DNA damage



Secondary screens: *arrayed CRISPR screens with high-throughput microscopy readout*

- Validation in 2 cell lines using 2 double-strand break inducing agents with different modes of action



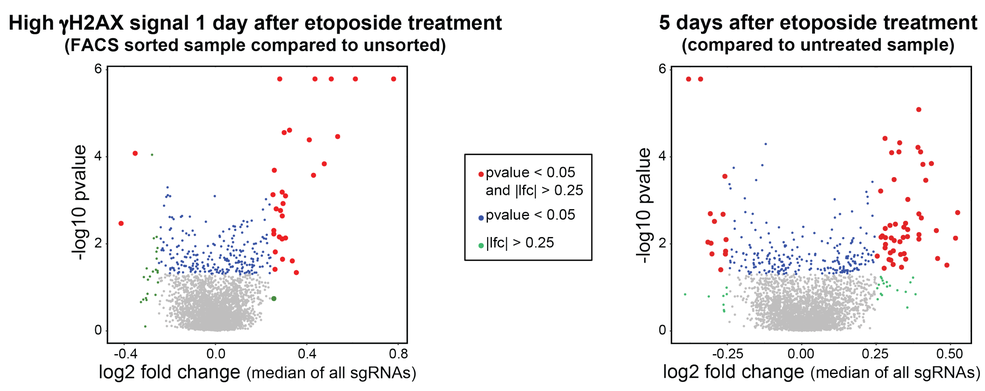
Conclusion and perspectives

This project sheds light on the essential crosstalk between cellular metabolism and the DNA damage response. Impairment of the pyrimidine *de novo* nucleotide synthesis pathway specifically generates endogenous DNA damage. Understanding in more details why only enzymes in the pyrimidine and not in the purine synthesis pathway have been identified by this experiment is currently under investigation.

Results

Primary screen

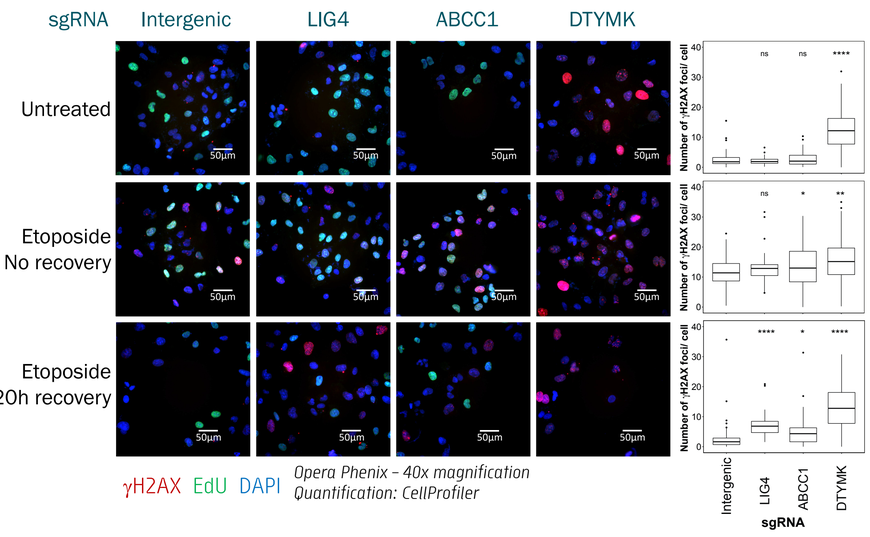
- sgRNAs targeting metabolic genes delay/ prevent DNA repair.
  - Persistent high γH2AX signal after recovery
  - Increased cell death after DNA damage



➢ **Metabolic genes impact on DNA repair following DNA double-strand break.**

Validation screens

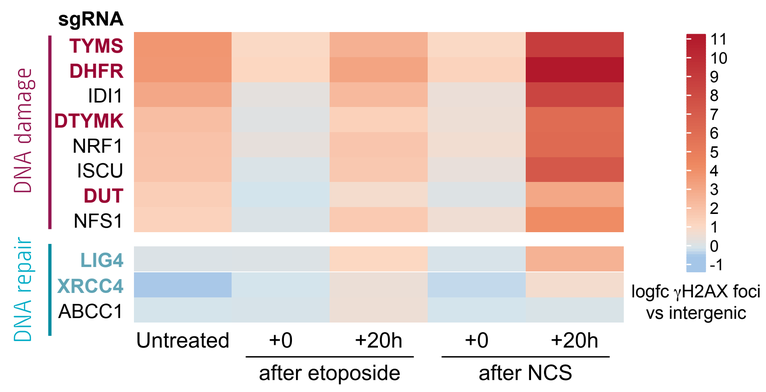
- Proteins identified during the initial screen and known to be involved in the DNA damage response have γH2AX kinetics consistent with their cellular functions.
- Depletion of enzymes involved in the pyrimidine deoxyribonucleotides *de novo* biosynthesis leads to the generation of DNA damage already at the basal level.



LIG4: Ligase 4, positive control
Non-homologous end-joining

ABCC1: ATP Binding Cassette Subfamily C Member 1
Transporter, involved in multi-drug resistance (efflux)

DTYMK: Deoxythymidylate Kinase
Pyrimidine deoxyribonucleotides de novo biosynthesis



TYMS, **DHFR**, **DTYMK** and **DUT** (in red) are involved in pyrimidine deoxyribonucleotides *de novo* biosynthesis.
Their depletion induces more γH2AX foci already in basal condition, indicating endogenous generation of DNA damage.

➢ **Depletion of metabolic proteins can both generate DNA damage (e.g. enzymes involved in pyrimidine *de novo* nucleotide synthesis) or delay DNA repair.**

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References

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