Targeted Single-Stranded and Double-Stranded DNA Breaks using CRISPR systems in Mammalian Cells

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BACKGROUND

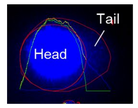
- Age is a risk factor for dementia, cardiovascular disease, osteoarthritis and cancer.
- DNA damage is a hallmark of aging and 10^4-10^5 damaging events occur each day.
- Accumulation of DNA damage can cause cell-cycle arrest or senescence.
- Induction of DNA damage for research has been done with chemicals or radiation.
- It is not possible to know number and location of DNA strand breaks with these methods.
- CRISPR is a gene editing tool that creates targeted DNA breaks.
- Previous studies have used CRISPR for creating double-stranded breaks (DSB) but no one to the best of our knowledge has studied single-stranded breaks (SSB).

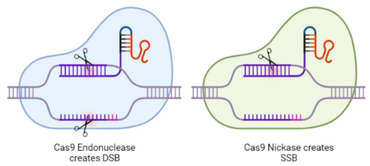
GOAL

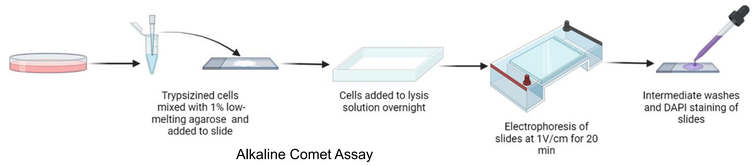
To use CRISPR systems with “promiscuous” crispr RNAs (crRNA) to generate single and double stranded cuts in the DNA and examine the impact of damage type on the detectable damage in the cell.

METHODS

- Alt-R CRISPR system (IDT) was transfected into mammalian cells using lipofectamine RNAiMAX with crRNAs targeting 50,150, or 1055 sites in the DNA. Cas9 endonuclease was used for DSB and nickase for SSB.
- DNA damage in cells was detected using Alkaline Comet Assay. Damaged DNA in comet tail cells was analyzed using ImageJ.







Alkaline Comet Assay

RESULTS AND DISCUSSION

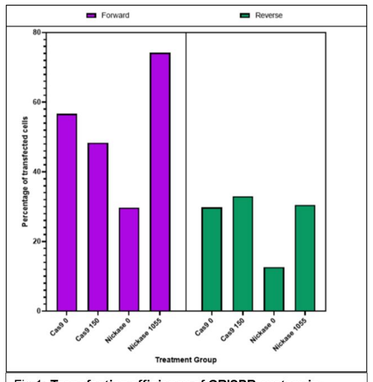


Fig 1: **Transfection efficiency of CRISPR system in synovial fibroblasts.** Percentage of transfected synovial fibroblasts was determined using flow cytometry with the ATTO 488 tracrRNA marker.

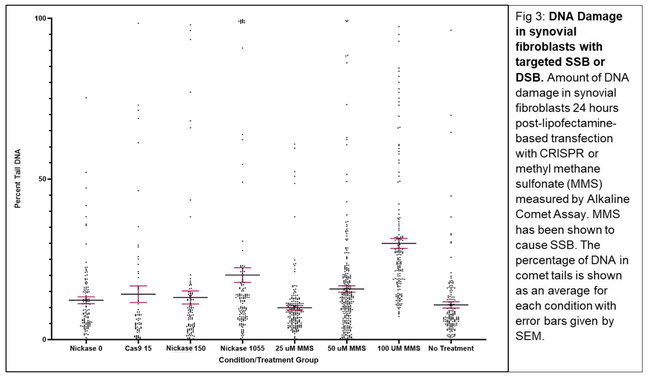


Fig 3: **DNA Damage in synovial fibroblasts with targeted SSB or DSB.** Amount of DNA damage in synovial fibroblasts 24 hours post-lipofectamine-based transfection with CRISPR or methyl methane sulfonate (MMS) measured by Alkaline Comet Assay. MMS has been shown to cause SSB. The percentage of DNA in comet tails is shown as an average for each condition with error bars given by SEM.

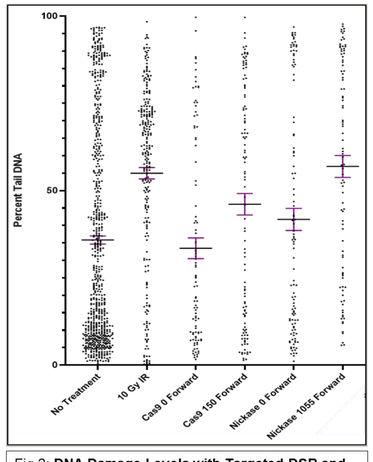


Fig 2: **DNA Damage Levels with Targeted DSB and SSB using CRISPR system in synovial fibroblasts.** DNA damage in synovial fibroblasts 36 hours post transfection detected by Alkaline Comet Assay which detects DSB and SSB simultaneously.

- More cuts in the DNA correspond to a detectable increase in the amount of nuclear damage in a cell using the Alkaline Comet Assay.
- 1055 SSB resulted in DNA damage comparable to that achieved through 10 Gy of radiation. 8 Gy of radiation kills 99% of people treated with same dose. This shows that the scale of DNA repair in humans is huge as we survive 10^4-10^5 DNA damaging events/day.
- The quantity of detectable damage in a cell is sensitive to the type of damage: SSB/DSB.

FUTURE DIRECTIONS

- Study DNA damage-driven senescence in cell lines that have markers for cell-cycle arrest and DNA replication to study the amount of damage required to trigger senescence and the timing in the cell-cycle
- Repetition of this study in cells from older donors to assess if CRISPR-induced DNA damage is detectable above background damage due to age.

CONCLUSION

- To understand aging better, DNA repair must be studied rigorously, which requires targeted means of causing and quantifying DNA damage.
- CRISPR is a valuable tool to create both SSB and DSB.

CLINICAL APPLICATIONS

CRISPR based targeted DNA damaging tools can be used to selectively kill cells that have mutations associated with cancer.

ACKNOWLEDGEMENTS

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