IMPACT OF G-QUADRUPLEXES ON DNA DOUBLE-STRAND BREAK REPAIR BY END JOINING

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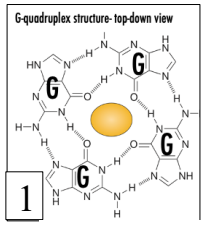
Introduction:

- G-quadruplexes (G4DNA), also called G-quartets, are hyper-stable secondary structures formed in guanine-rich DNA sequences (fig 1).[1]
- About 300,000 G4DNAs exist in the human genome, often in critical regions like telomeres and promoters. It has been shown to drive genomic instability by impeding DNA replication and some forms of DNA repair.[2]



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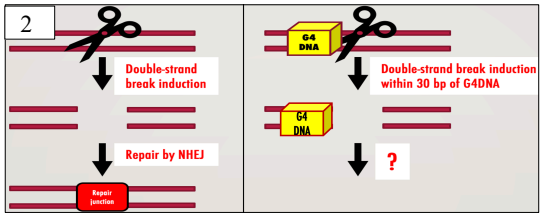
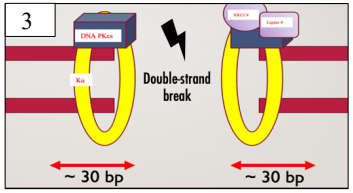
Graphical Abstract:

- Deficiencies in DNA double-strand break (DSB) repair by non-homologous end joining (NHEJ) contribute to genomic instability, the impact of G4DNA on this process is unknown (fig 2).



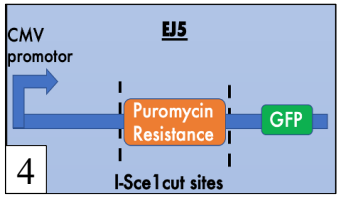
Hypothesis:

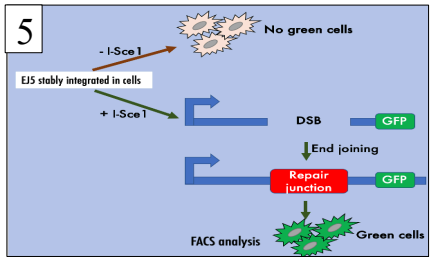
- NHEJ requires ~30bp on both sides of the DSB for assembly of repair factors (fig 3).[3] We hypothesized that presence of G4DNA within that 30bp will obstruct assembly and negatively impact repair by NHEJ.



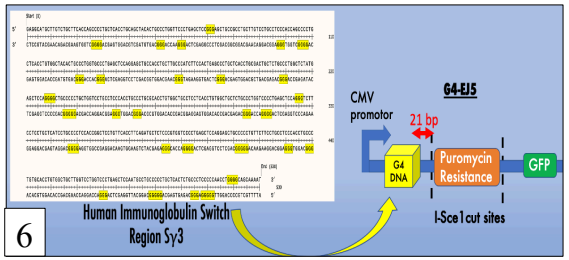
Experimental design:

- We tested our hypothesis with the EJ5 NHEJ reporter system in which repair of an I-SceI-generated DSB within the EJ5 sequence (fig 4) permits GFP expression (fig 5).

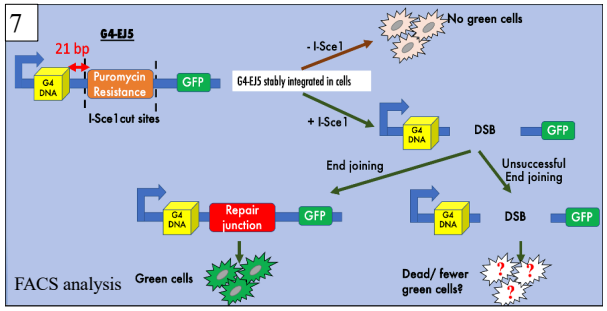




- A G4DNA-forming sequence was inserted 21 bp upstream of the I-SceI site to create G4-EJ5 (fig 6).
- Cell lines with integrated EJ5 or G4-EJ5 were then produced through stable transfection into human HEK293 cells.

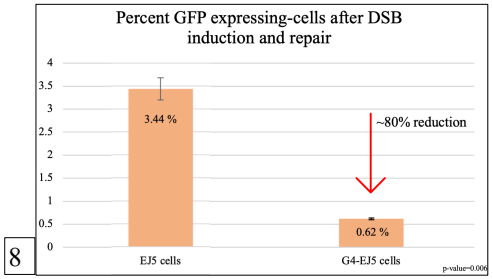


- Transient expression of I-SceI produced DSBs, and repair by NHEJ permitted GFP expression, which was measured using flow cytometry. (fig 7).



Preliminary results:

- Presence of G4DNA in G4-EJ5 was confirmed by PCR analysis. Our preliminary data show an 80% reduction in GFP expression in G4-EJ5 cells as compared to EJ5 cells (fig 8). Results show statistical significance with p-value ≤ 0.01



Conclusion:

- Decrease in GFP expression in G4-EJ5 cells indicates decrease in end joining repair efficiency, most likely due to the presence of G4DNA near the DSB site. Our immediate goal is to compare NHEJ fidelity between EJ5 and G4-EJ5 cells.

Future Directions:

- Testing our hypothesis using a different G4DNA-forming sequence, to assess possible inter-sequence variation.
- Despite their unusual structure and stability, G4DNA may be removed by specific helicases and nucleases. We plan to assess the role of these enzymes and the impact of G4DNA-stabilizing drugs on NHEJ.

References:

1. Hertsch et al., *Nat Rev Mol Cell Biol*. 2017.
2. Rhodes et al., *Nucleic Acids Res.* 2015
3. Walker et al., *Nature* 2001

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