The Role of the SAGA Histone Acetyltransferase and Core Structural Module in DNA Damage Repair

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Introduction

The Spt-Ada-Gcn5 acetyltransferase (SAGA) complex is a highly-conserved transcriptional coactivator, with its multiple modules each serving a distinct function. Alongside a core structural module, SAGA contains a histone deubiquitinase (DUB) module and histone acetyltransferase (HAT) module that modify chromatin and contribute to transcriptional regulation. Recent findings from the Wilkinson lab identified the SAGA subunits Tada2b (member of the HAT module) and Taf5l (member of the core structural module) as key regulators of lineage commitment and ageing in haematopoietic stem cells (HSCs) (Haney et al., bioRxiv, 2022). However, the mechanism remains unclear.

Recent findings have also shown that the SAGA DUB module affects DNA double-strand break (DSB) damage repair signalling (Ramachandran et al., 2016). When key subunits are knocked out, the loss of H2BK120ub deubiquitylation activity will interfere with proper γH2AX formation and DSB repair. Given the high cooperation and coordination between the SAGA modules, we hypothesised that the other modules also play a role in DSB repair. Here, assays utilising immunoglobulin class switch recombination, which utilises NHEJ, act as a proxy to test for defects in DSB repair.

Aims

1. Ensure that γH2AX is formed when DSBs are present in murine haematopoietic stem and progenitor cells (HSPCs).
2. Observe the effects of CRISPR knockout of key subunits in the SAGA HAT and core structural module on class switch recombination.

Methods

- **DNA damage induction and HSC kill curve**

Mouse bone marrow cKit+ HSPCs were extracted and cultured. The topoisomerase I inhibitor camptothecin and the topoisomerase II inhibitor etoposide were then titrated to identify the optimum concentration to cause DSBs without being lethal.

- **Intracellular Staining for γH2AX**

50nM of camptothecin and 100nM of etoposide were separately added to extracted HSCs. The cells were then fixed and permeabilized before anti-γH2AX antibody was added. Flow cytometry was used to identify the population of γH2AX+ cells.

- **CRISPR knockout of SAGA complex subunits**

Ribonucleoproteins (RNPs) of sgRNAs with Cas9 were introduced into CH12 (murine B-cell lymphoma) cells via electroporation. The resulting cells were serially diluted to grow single-cell clones from which we selected those with a homozygous KO genotype.

- **PCR and Sanger sequencing for identification of homozygous CH12 knockout (KO) clones**

Samples from the clonal cultures were lysed for PCR with primers to amplify the edited region. The PCR product was purified and sent for Sanger sequencing. The results were analysed using the CRISPR ICE analysis tool (Synthego).

- **Class Switch Recombination Assay (CSA)**

Homozygous KO CH12 clones were stimulated by anti-CD40 antibody, TGFβ and IL4 to induce class switching from the isotype IgM to IgA. After ~48h, the cells were stained with anti-IgA and assessed by flow cytometry to determine the frequency of IgA+ cells.

Results and Outcomes

The γH2AX intracellular staining does show a population of γH2AX+ cells which is larger for cells exposed to 50nM camptothecin, which was initially identified as the more lethal treatment. This shows that γH2AX formation does follow induction of DSBs in HSPCs *(Fig. 1)*.

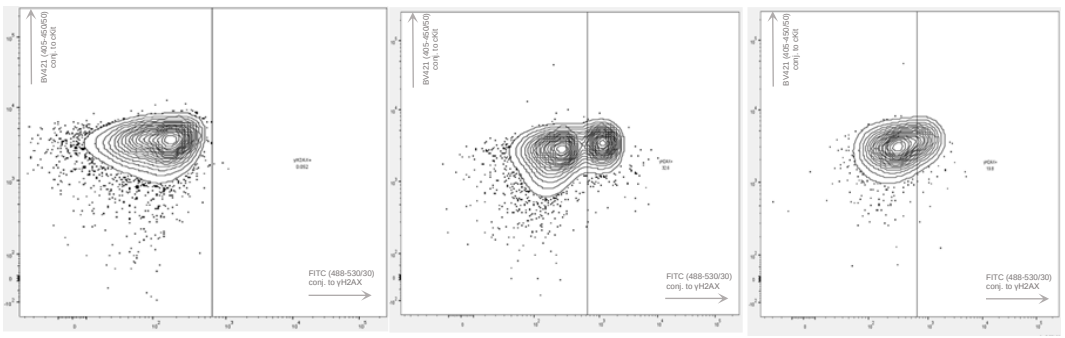

Figure 1: Flow cytometry plot of FITC (conj. to anti- γH2AX) fluorescence against BV421 (conj. to anti-cKit) fluorescence. Cells untreated with DNA damaging agents did not show any γH2AX, while cells treated with camptothecin at 50nM (centre) and etoposide at 100nM (left) both showed a clear population of γH2AX+ cells.

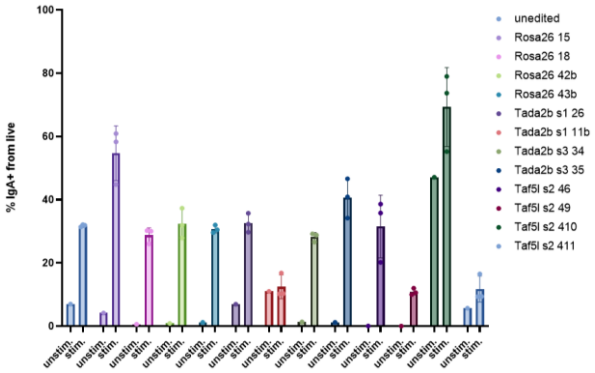

Figure 2: CSA result which includes all obtained clones. Some clones (Rosa26 18, Tada2b 11b, Taf5l 410) show an anomalous phenotype and are excluded from analysis.

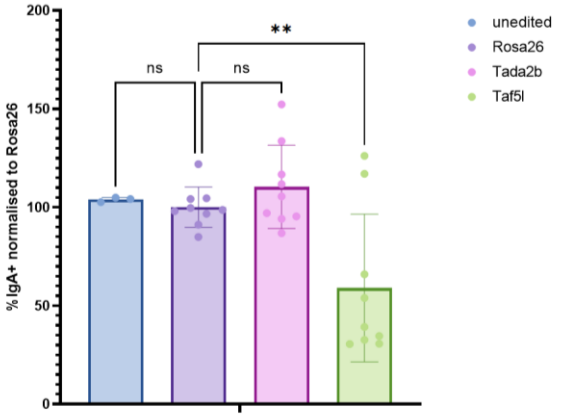

Figure 3: Final CSA results which is normalised to the control clones for Rosa26. Clones of the same gene knockout were grouped together. Anomalous results were excluded from the calculations. One-way ANOVA was run to test for significance.

CRISPR KOs were performed using sgRNAs targeting genes for Rosa26 (a genomic safe harbour, acts as a control), Tada2b (from HAT module), Taf5l and Taf6l (from core structural module). Altogether, we managed to obtain four homozygous KO clones for each gene except Taf6l, which we only derived one. Multiple CSA experiments suggested that while Tada2b KO does not show an effect on class switching, Taf5l KO likely does *(Fig. 3)*. While the limited data collected for Taf6l KO does suggest a potential effect, it is currently inconclusive.

Unfortunately, after continuous passages, the CH12 cells started showing abnormal phenotypes and behaviour in culture. These clones were excluded from the data analysis.

Discussion and Future Directions

We found that the SAGA core structural module might be involved in DNA damage repair as Taf5l KO significantly impairs class switch recombination *(Fig. 3)*. Considering the role of the core structural module in mediating interactions and coordination between the other modules, along with the findings from Ramachandran et al., 2016, the effect may be due to interference in the activity of the DUB module. Better understanding of the involvement of the SAGA complex in the DSB repair in HSCs will help improve the current perspective on the processes underlying HSC ageing and the development of haematological malignancies.

The emergence of abnormal phenotypes in culture was an unfortunate circumstance most likely due to a bad batch of CH12 cells that was used to generate the KO clones. The inherent genomic instability of cancer cells coupled with the harsh conditions required to form KO clones likely caused unwanted mutations that altered the phenotype. Repeats of this experiment with a batch of early passage CH12 cells while avoiding prolonged culture will likely solve this problem.

The future of this project likely involves targeting different SAGA subunits for CRISPR KO, particularly the DUB and HAT catalytic subunits Usp22 and Kat2a. CRISPR KOs can also be done in primary HSCs, with DSB repair reporter plasmids being used in lieu of class switching. Expanding on the role of γH2AX, intracellular staining for γH2AX and H2BK120ub after CRISPR KO of key SAGA subunits would help elucidate the mechanism behind the observed defect on DSB repair.

Value of Studentship

To student: Through this studentship, I have not only learnt an immense array of experimental techniques (e.g. mammalian cell culture, flow cytometry, PCR etc.) but also the bioinformatics which precede and follow them. Furthermore, having first-hand experience in scientific research with all its ups and downs helped me hone my critical thinking to solve problems and form hypotheses. The lab was also very friendly and helped me through discussions and some great advice.

In order from left to right: Ms. Grace Meaker, Ethan Sip, Dr. Leonid Olender and Dr. Adam Wilkinson

To lab: Hosting Ethan via this studentship was a great opportunity for our lab to contribute to the training of a new generation of scientists. It was also excellent for the development of leadership and mentorship skills. Ethan took responsibility for driving forward this research project, performed experiments independently and to a high standard, and made impressive progress over a short period of time. His work has helped to shape the future direction of our research into the role of SAGA in HSCs.

References:

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