OGT prevents DNA demethylation and suppresses the expression of transposable elements in heterochromatin by restraining TET activity genome-wide

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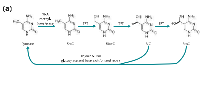
1. Introduction

We present a study investigating the effect of deletion of the epigenetic regulation enzyme O-GlcNAc transferase (OGT) on genome-wide 5mC and 5hmC levels in mouse embryonic stem cells (mESC) using a novel 6-base sequencing technology.

The O-GlcNAc transferase OGT interacts robustly with all three mammalian Ten-Eleven Translocation (TET) methylcytosine dioxygenases. Using duet evoC 6-base sequencing (enabling individual discrimination of A, C, T, G, 5mC and 5hmC), we show that deletion of the Ogt gene in mESC results in a widespread increase in the TET product 5hmC in both euchromatic and heterochromatic compartments, with concomitant reduction of the TET substrate 5mC at the same genomic regions. mESC treated with an OGT inhibitor also displayed increased 5hmC demonstrating OGT enzymatic activity is needed to suppress TET activity. This indicates that OGT restrains TET activity and limits untoward DNA demethylation in a manner that requires the TET-OGT interaction and the catalytic activity of OGT. DNA hypomethylation in OGT-deficient cells was accompanied by de-repression of transposable elements (TEs) predominantly located in heterochromatin.

We suggest that OGT protects the genome against TET-mediated DNA demethylation and loss of heterochromatin integrity, preventing the aberrant increase in TE expression noted in cancer, autoimmune-inflammatory diseases, cellular senescence and ageing.

2. duet evoC 6-base sequencing [A,C,T,G,5mC,5hmC]



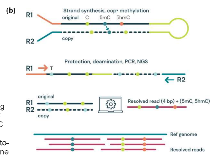
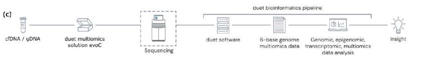


Figure 1. (a) TET-mediated demethylation pathway
(b) duet multiomics solution evoC - a 6-base sequencing technology that reads all four canonical bases plus 5mC and 5hmC[1] via strand copy, 5mC copy and 5mC + 5hmC protection enzymatic steps.
(c) The duet multiomics solution evoC works as an end-to-end solution comprising reagents & bioinformatics pipeline



3. OGT deletion decreases 5mC and increases 5hmC across the genome

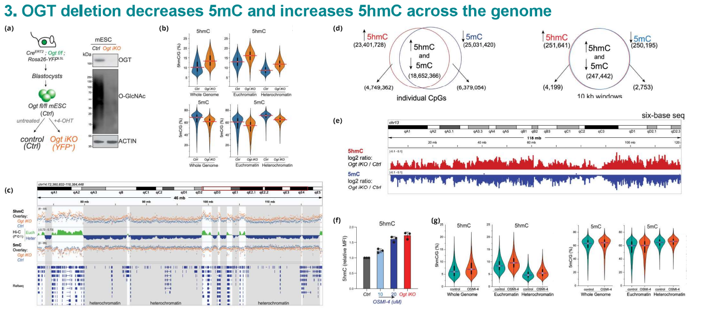
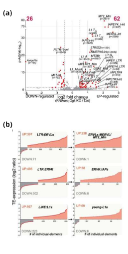

(a)
Cre^ERT2 ; Ogt f/f ; Rosa26-YFP^LSL
Blastocysts
Ogt fl/fl mESC (Ctrl)
untreated
+4-OHT
control (Ctrl)
Ogt iKO (YFP+)
mESC
Ctrl
Ogt iKO
kDa
OGT
O-GlcNAc
ACTIN
(b)
5hmC
5mC
Whole Genome
Euchromatin
Heterochromatin
(d)
5hmC (23,401,728)
5mC (25,031,420)
5hmC and 5mC (18,652,366)
(4,749,362)
(6,379,054)
individual CpGs
5hmC (251,641)
5mC (250,195)
5hmC and 5mC (247,442)
(4,199)
(2,753)
10 kb windows
(e)
six-base seq
5hmC log2 ratio: Ogt iKO / Ctrl
5mC log2 ratio: Ogt iKO / Ctrl
(c)
5hmC Overlay: Ogt iKO Ctrl
Hi-C (PC1) Euch Heter
5mC Overlay: Ogt iKO Ctrl
Refseq
heterochromatin
(f)
5hmC
5hmC (relative MFI)
Ctrl
10
20
Ogt iKO
OSMI-4 (uM)
(g)
5hmC
5mC
control
OSMI-4
Whole Genome
Euchromatin
Heterochromatin

Figure 2. (a) Left. Generation of *Ogt iKO* mESC. Right. Western blots showing loss of OGT protein (*top*) and *O*-GlcNAc modification (*bot*) in whole-cell lysates.
(b) 6-base sequencing shows increased 5hmC (*top*) and decreased 5mC (*bot*) in *Ogt iKO* compared to *Ogt fl (Ctrl)* mESC at whole genome resolution (*left*) or chromatin compartments (*right*).
(c) Genome browser view of 5hmC (*top*) and 5mC (*bottom*) in overlaid tracks of *Ogt iKO (orange)* and *Ctrl Ogt fl (blue)* mESC. 10 kb windows averaged. Euchromatin (*green*), heterochromatin (*blue*).
(d) Venn diagrams showing overlap between gaining 5hmC (red) and losing 5mC (blue) in *Ogt iKO* mESC vs *Ctrl Ogt fl* mESC for individual CpGs left or 10kb windows right.
(e) Genome browser view showing reciprocal gain of 5hmC (*red*) and loss of 5mC (*blue*) in *Ogt iKO* vs *Ctrl Ogt fl* mESC. Plotted: log2 ratios (*Ogt iKO/ Ctrl Ogt fl*) for 5hmC (*top*) and 5mC (*bottom*).
(f) Increase of 5hmC after OGT inhibition with OSMI-4. 5hmC measured by flow cytometry after 4 days of OSMI-4 treatment. Bar graphs represent the mean ± s.d. from three independent experiments.
(g) Violin plots showing increased 5hmC levels (left) but unaltered 5mC levels (right - likely due to earlier earlier growth arrest) in OSMI-4-treated versus control mES cells.

3. Decreased 5mC and increased 5hmC result in increased TE expression



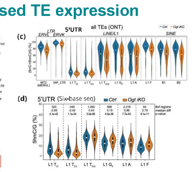
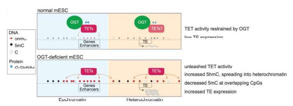


Figure 3. (a) Volcano plot of RNA-seq data showing differential TE expression in *Ogt* iKO vs Ctrl *Ogt fl* mESC. Red dots: transcripts exhibiting ≥ twofold expression change.
(b) RNA-seq data of *Ogt* iKO vs Ctrl *Ogt fl* mES cells, analysed for differential expression of uniquely mapped TEs. No. of upregulated (red) & downregulated (gray) TEs.
(c) modC (5mC+5hmC) levels of TE subfamilies in *Ogt fl* (blue) vs *Ogt* iKO (orange) mES cells (ONT)
(d) 5hmC levels at 5′ UTRs of indicated L1 families in Ctrl *Ogt fl* and *Ogt* iKO mES cells; difference of median 5hmC levels between conditions (*Ogt* iKO − Ctrl) displayed on top

4. Conclusion

We report here that OGT globally restrains TET enzymatic activity and maintains DNA methylation genome-wide, in a manner that depends on OGT catalytic activity and the TET–OGT interaction (illustrated below). TET activity was unleashed upon *Ogt* gene deletion or OGT inhibition, resulting in ongoing demethylation defined by parallel increases in 5hmC and decreases in 5mC at overlapping CpGs, observed using duet multiomics solution evoC 6-base sequencing. The discovery (in press[2]) reveals a novel mechanism for maintaining genomic stability, with important implications for both development and disease.


normal mESC
OGT
TETs
TETs?
Genes Enhancers
TE
TET activity restrained by OGT
DNA
5mC
C
Protein
OGT-deficient mESC
unleashed TET activity
increased 5hmC, spreading into heterochromatin
decreased 5mC at overlapping CpGs
increased TE expression

5. References

1. Füllgrabe J. et al. Simultaneous sequencing of genetic and epigenetic bases in DNA. Nat Biotechnol. 2023 Oct;41(10):1457-1464.
2. Sepulveda, H. et al. OGT prevents DNA demethylation and suppresses the expression of transposable elements in heterochromatin by restraining TET activity genome-wide. Nat. Struct. Mol. Biol. (2025 - in press)

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