Epigenetic Consequences of BCG-activated Leukocytes on Bladder Cancer Cells

Lucy C. Picard[1], Fenella Rich[1], Logan C. Walker[2], Aaron J. Stevens[1].
[1]Pathology and Molecular Medicine, UO Wellington, [2]Pathology and Biomedical Science, UO Christchurch

Department of Pathology and Molecular Medicine

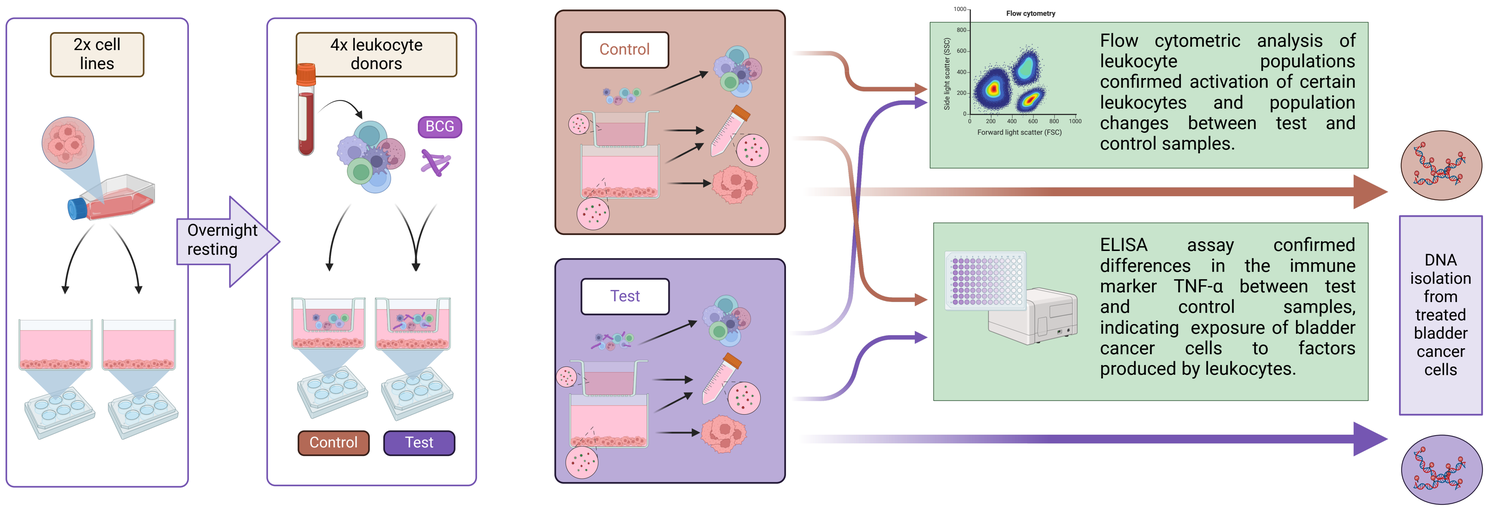
INTRODUCTION

Immune cells, especially when activated, have the ability to change the microenvironment of cancer via cytokines and reactive oxygen species in a way that could influence epigenetic mechanisms of surrounding cells. Bacillus Calmette-Guerin (BCG) mycobacteria is a well established treatment of non-muscle invasive bladder cancer. Potential epigenetic consequences of this treatment were investigated in this research using an *in vitro* co-culture system, combining leukocytes, BCG, and bladder cancer cells. Two cell lines were used: SW780 (S) and RT4 (R).

OBJECTIVE

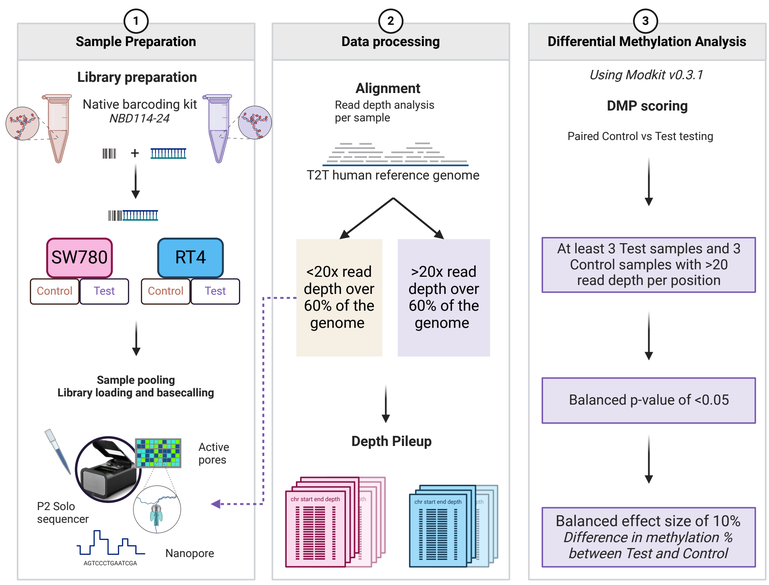
To profile changes in cytosine and adenine DNA methylation between cancer cells co-cultured with activated vs non-activated leukocytes. Whole genome DNA sequencing and DNA base modifications were determined using the PromethION 2 Solo nanopore sequencing device.

METHODS



Bladder cancer cell lines were split into treatment and control plates. Isolated leukocytes were added to co-culture and either activate with BCG or treated with PBS. Each sample was added into all upper compartments of the transwell plate system. Leukocytes, BCG and bladder cancer cells were cultured for two cell divisions: 48 hours for SW780 (S), 72 hours for RT4 (R)

Leukocytes, both in suspension and adherent to the transwell membrane, were harvested and prepared for flow cytometric analysis. Media from both compartments was isolated and BCG eliminated before preparation for ELISA assay. Cancer cells were harvested and prepared for DNA extraction.



DNA sample preparation was carried out using the native barcoding protocol (Oxford Nanopore Technologies). Sequence data were aligned to the T2T human reference genome. Positions used to calculate differential modification scores were included only if they had >=20x read depth. "Balanced" values indicate adjustments for sample size used as per Modkit's description. More information can be found by following the QR code.

RESULTS

Flow cytometry analysis of leukocytes confirmed a change in phenotype and activation between test and control samples. The pattern of activation was slightly different between each healthy participant, and this differing response likely determines what was observed in bladder cancer cell response. The current sample size is, however, insufficient to fully understand these variations, and is outside the scope of this investigation.

Cell Line	Base with modification: C	Base with modification: A
SW780	59	28
	0	0
RT4	61	17

Table 1: Number of base modifications per C/A base per cell line.

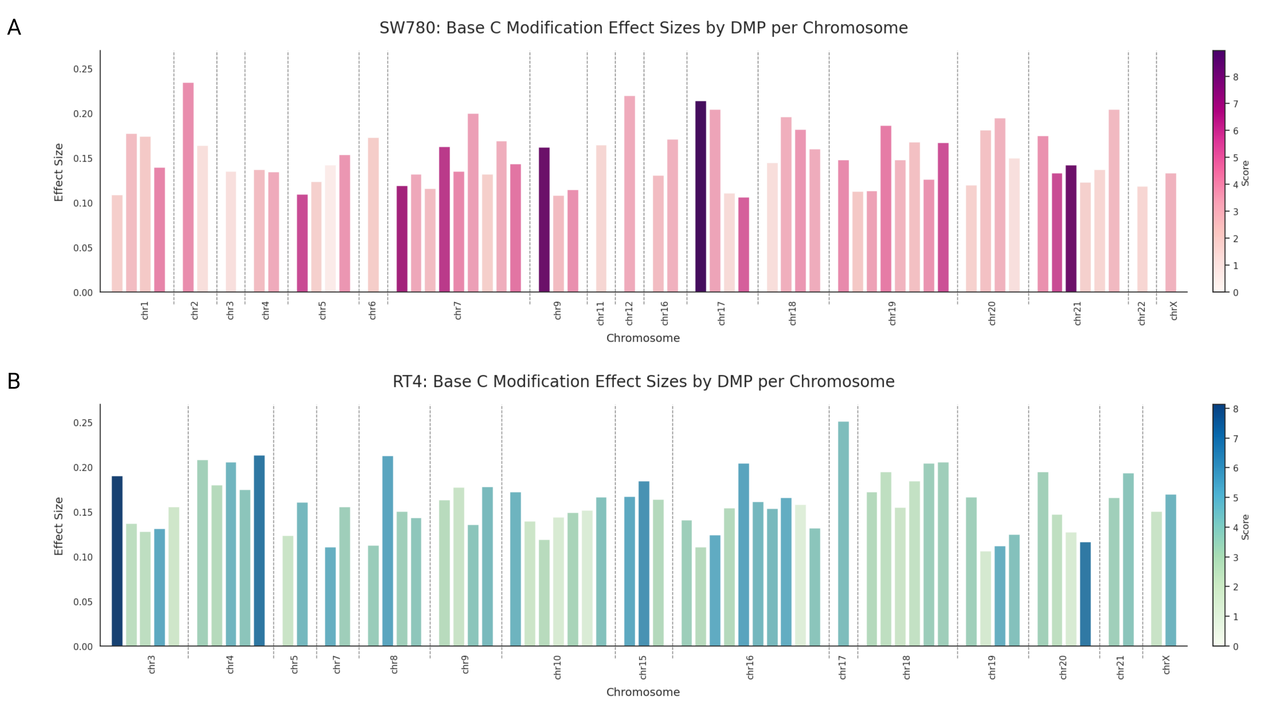


Figure 1: Differentially methylated base C positions identified in cell line SW780 (**A**) and RT4 **(B),** plotted by chromosome position against effect size, and coloured by significance score (higher values mean more significant results). P-value threshold >=0.05, effect size threshold >=10%.

Figure 2: Differentially methylated base A positions identified in cell line SW780 (**A**) and RT4 (**B**), plotted by chromosome position against effect size, and coloured by significance score (higher values mean more significant results). P-value threshold >=0.05, effect size threshold >=10%.

Sequencing data was aligned to the recently published Telomere-to-Telomere human genome assembly. There were 87 and 78 DMPs identified on SW780 and RT4 cell lines respectively, none of which were in common (see Table 1).
Figure 1 and figure 2 show differentially methylated positions (DMP) along the genome per cell line, at bases C and A respectively, plotted by effect size (the difference in percent methylation of positions between paired test and control samples).
Differential modification appears to be cell line specific, as there were no significant DMPs common between cell lines.
There appears to be a high concentration of DMPs occurring at centromeres (see data linked in QR code).
Further verification of these results is needed, as varying read depth may influence significance scoring. Additional investigation into functional locations of identified DMPs is ongoing, as the T2T genome lacks robust annotations at this time.

CONCLUSION

Leukocyte response to BCG exposure is associated with changes in cytosine and adenine base modifications in adjacent cancer cells. This occurs without physical contact between immune and cancer cells.
This likely has implications for understanding patient response to BCG during bladder cancer treatment and highlights the highly context dependent nature of the interactions.

Further research is required to establish the biological implications of these changes.

ACKNOWLEDGEMENTS

This work was funded by the Health Research Committee of NZ and the Otago Doctoral Scholarship.

REFERENCES

1. Das, A. B., Seddon, A. R., O'Connor, K. M. & Hampton, M. B. Regulation of the epigenetic landscape by immune cell oxidants. Free Radical Biology and Medicine 170, 131–149 (2021).
2. Scelfo, A. & Fachinetti, D. Keeping the Centromere under Control: A Promising Role for DNA Methylation. Cells 8, 912 (2019).
3. Picard, L. C., Rich, F. J., Kenwright, D. N. & Stevens, A. J. Epigenetic changes associated with Bacillus Calmette-Guerin (BCG) treatment in bladder cancer. Biochimica et Biophysica Acta (BBA) - Reviews on Cancer 1879, 189123 (2024).