What are the changes in gene expression between IR and non-IR tumours at 24 hours and 7 days in the A20 mouse model?

In the study by Kostopoulos et al. (2024), the researchers explore how low dose radiation therapy enhances the efficacy of CAR T-cell therapy for CD19+ lymphomas. To perform their study, the authors used 6-8 week old female mice that were injected with A20 lymphoma cells on each side of their lower back. The tumours were left to grow for 19 days, and one side of each mouse was irradiated while the other was not. After 24 hours, gene expression was measured on both the irradiated (IR) and non-irradiated (non-IR) sides, and then once more 7 days post treatment (Supplementary Figure 1). The researchers found that 1-2 fractions of low-dose RT improved tumour control at the irradiated site, and exhibited a positive effect on generating abscopal systemic antitumor responses beyond the tumour sites. Their findings suggest activation of immune pathways and the spreading of antigens to be the mechanism of therapy. Ultimately, their results show that low-dose RT can be effective for ameliorating CAR T-cell therapy and reducing lymphoma treatment resistance.

The methods and findings of this article led us to explore the following question:

What are the changes in gene expression between IR and non-IR tumours at 24 hours and 7 days in the A20 mouse model?

To conduct our analysis, we retrieved the raw data from the authors of this study and began with data wrangling. Our first steps in analysing the data was installing and loading the required libraries. We used both base R (head, colnames, str, summary, anyNA) and library specific (glimpse) functions to learn about the data.

From our data wrangling analysis, we observed that our dataset had 49763 rows (genes) and 8 columns (samples). The gene expression data was numerical with no NAs and no negative values. The minimum gene expression data value was 0, meaning no expression, and the maximum was 243679. All 8 samples had similar expression summaries. For example, for sample “NK6809” the third quartile was 58, meaning 75% of our data had a gene expression value that was less or equal than 58. On average, 35% to 47% of the data had no gene expression (gene expression = 0), whilst 53% to 65% showed a gene expression greater than 0 (gene expression != 0).

To answer our question we chose 8 samples, 2 from each experimental condition. We performed differential expression analysis using raw data as required by the DESeq library. To create our histogram we used the pivot_longer function and filtered for expression 0>x>100 to illustrate the data distribution (Supplementary figure 2). We found that most of the genes with detected expression had an expression range between 1 and 3.

Subsequently, we aimed to create a PCA plot to assess clustering amongst the variables and to gain insight into any relationship amongst them (Supplementary Figure 3). However, the plot showed little clustering, which can be attributed to the use of the raw count data instead of normalized data for the PCA.

Differential expression analysis (DEA) was then used to compare the expression profiles of the various conditions (Figure 1). The DEA shows that after 24 hours there is little change between both groups (Figure 1.a). However, gene expression is most upregulated and statistically significant in the irradiated group 7 days post treatment, compared to the non-irradiated group (Figure 1.b, Supplementary Figure 4).To gain further insight into the biological significance of the difference in expression profiles between the IR and non-IR tumors after 7 days, gene set enrichment analysis was performed (GSEA). The results of the GSEA show that pathways related to metabolic stress response, neuronal processes, and immune modulation were the most active during treatment (Figure 2).

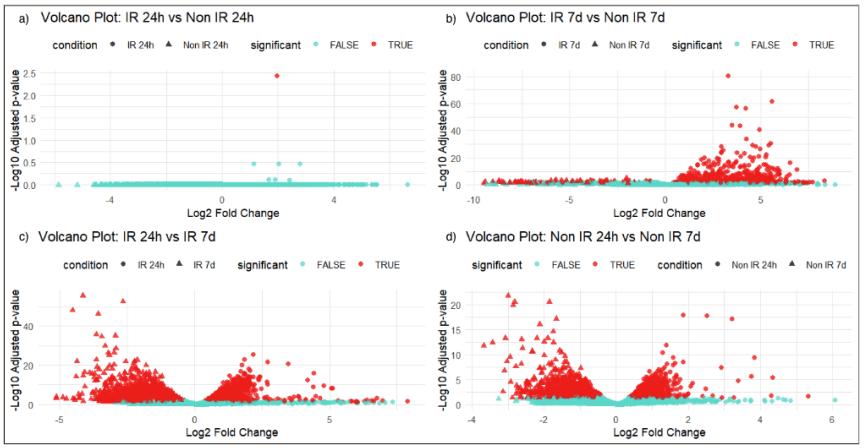


Figure 1: Volcano plots of Log2fold change between expression of different treatment groups between different time periods. a) Comparison of log2fold change between irradiated and non-irradiated groups at 24h. b) Comparison of log2fold change between irradiated and non-irradiated groups after 7 days. c) Comparison of log2fold change between irradiated groups at 24 hours and 7 days. d) Comparison of log2fold change between non-irradiated at 24 hours and 7 days.

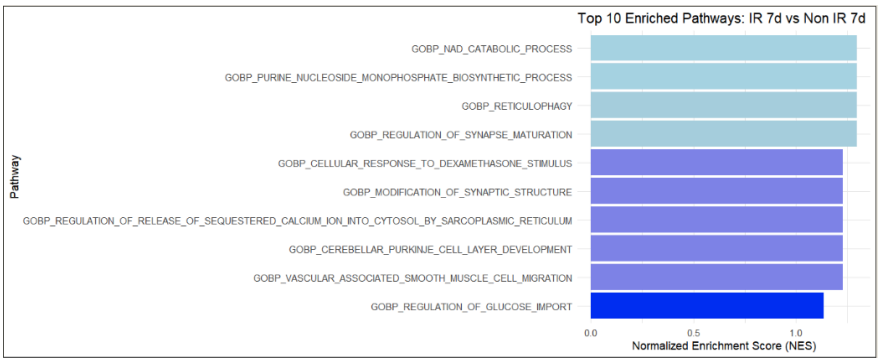
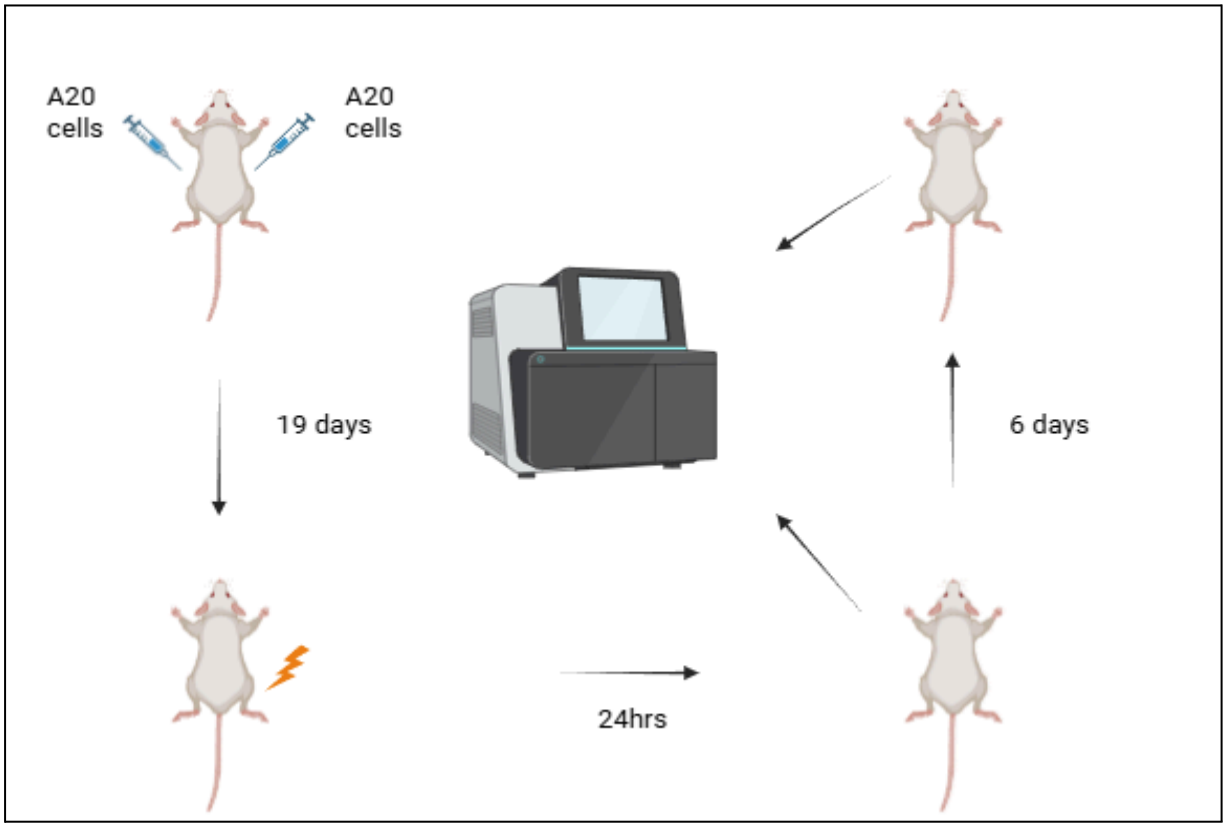


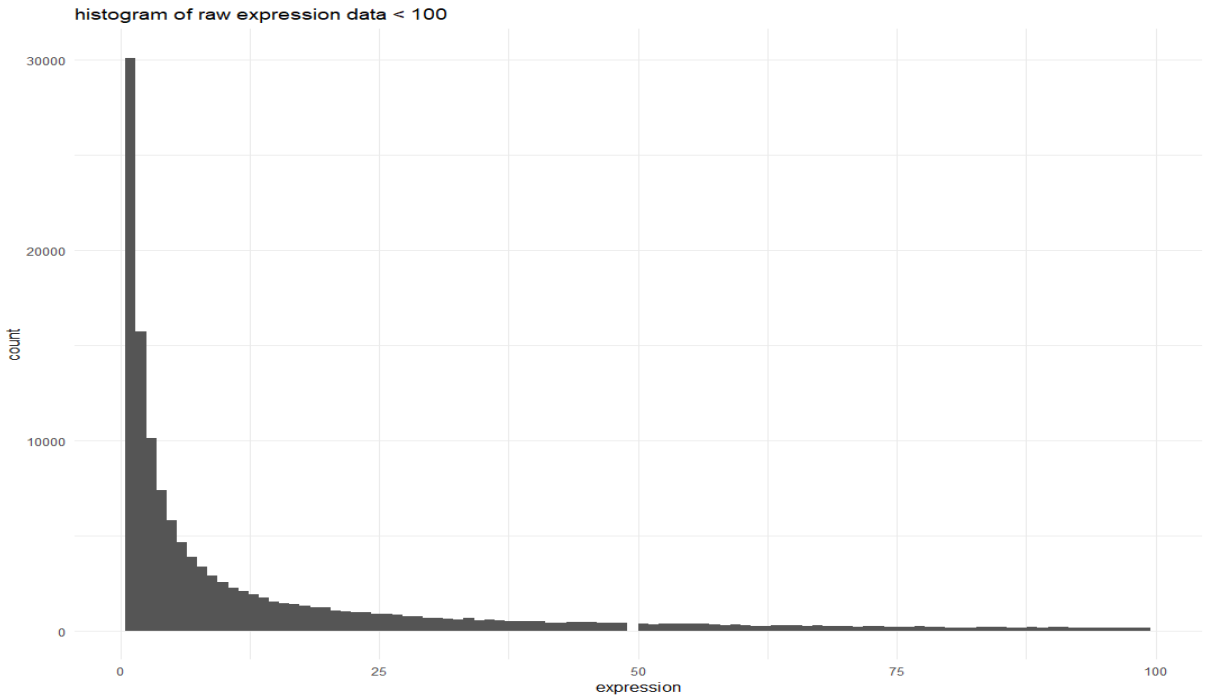
Figure 2: Gene set and pathway enrichment analysis showing the most enriched pathways.

From our analysis, we were able to see little to no change between gene expression in the IR and non-IR models after the first 24 hours. After 7 days however, there were visible changes between gene expression in the IR and non-IR tumours (Figure 1). The changes being a strong upregulation in gene expression in the irradiated models, and less expression in the non-irradiated tumours (Supplementary Figure 4). This suggests that radiation elicits effects which are delayed. The top pathways that were affected by radiation were involved in metabolic stress response, immune modulation, and neuronal processes (Figure 2).

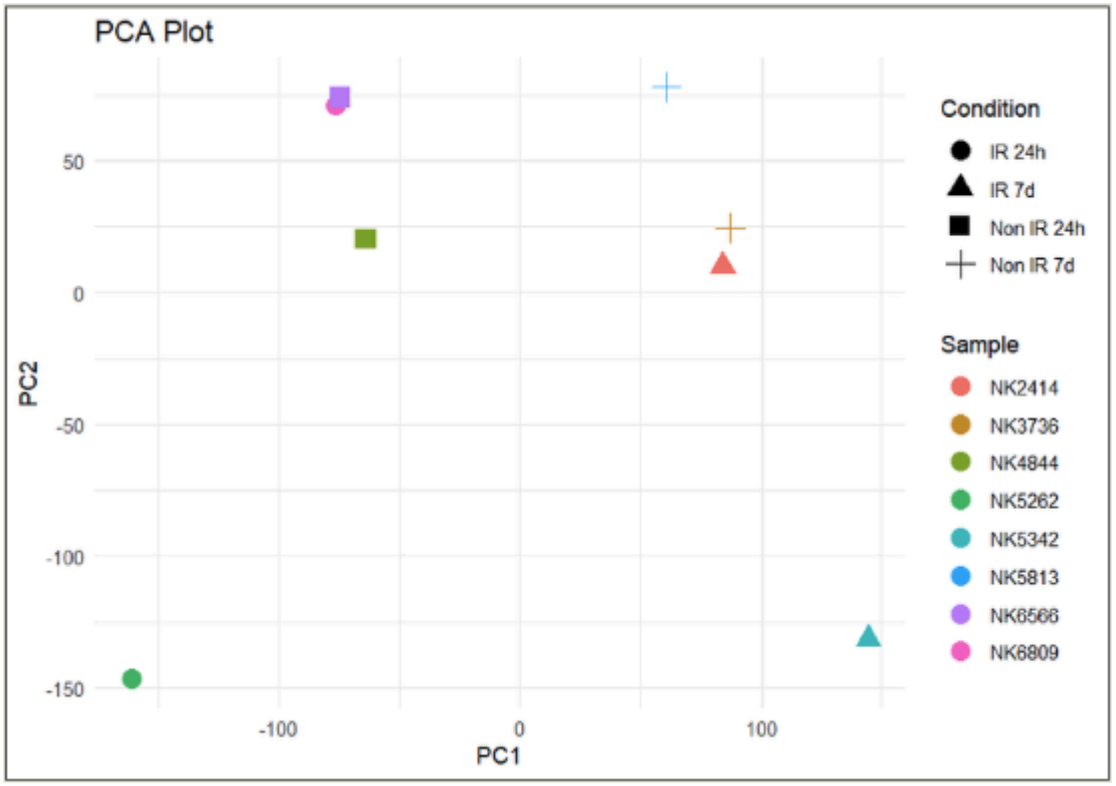
Supplementary Material



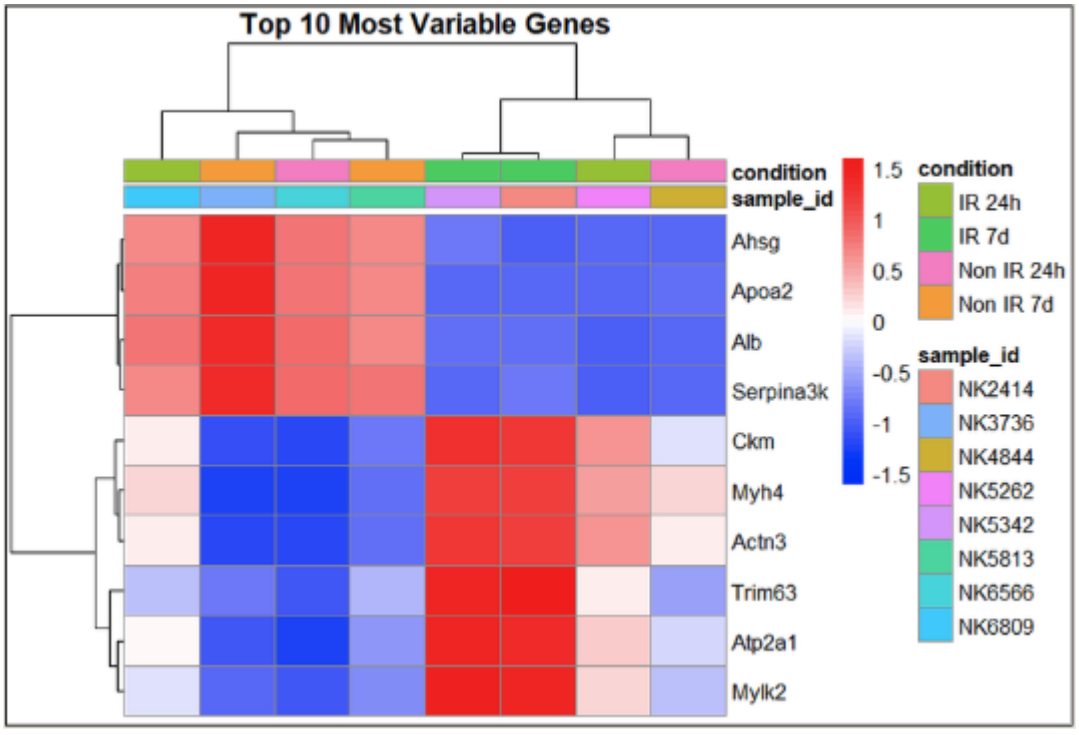
Supplementary Figure 1: Summary of Methodology. Mice were injected bilaterally with A20 cells. Tumors were left to grow for 19 days. One tumor was irradiated with 8Gy. Gene expression was measured after 24 hours and then again after 7 days.



Supplementary Figure 2: Histogram of raw expression data.



Supplementary Figure 3: PCA plot of raw expression data.



Supplementary Figure 4: Heat map of top 10 most variable genes.

References

Kostopoulos, N., Costabile, F., Krimitza, E., Beghi, S., Goia, D., Perales-Linares, R., Thyfronitis, G., LaRiviere, M. J., Chong, E. A., Schuster, S. J., Maity, A., Koumenis, C., Plastaras, J. P., & Facciabene, A. (2024). Local radiation enhances systemic CAR T cell efficacy by augmenting antigen Cross-Presentation and T-cell infiltration. *Blood Advances*. https://doi.org/10.1182/bloodadvances.2024012599

Source code: github (https://github.com/annalifousi/gene-expression-analysis)
Data: GSE281695 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE281695)