Investigating the Effects of PU.1 and CEBPα Co-transfection on B16BL6 Melanoma Cells

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Introduction

- Macrophages are innate immune cells that phagocytose microbes
- In addition, macrophages can polarize into proinflammatory M1 macrophages which arise in response to toll like receptor (TLR) signaling by ligands such as lipopolysaccharide (LPS) - a component of gram-negative bacteria cell wall
- Macrophage polarization is needed to mediate appropriate responses in response to various tissue microenvironments
- Previous work in the lab on the murine macrophage RAW264.7 cell line showed that prior administration of a MEK inhibitor (U0126) primes the inflammatory response following exposure to LPS compared to samples that did not receive the U0126 treatment before LPS exposure – this priming effect resulted in the increased expression of pro-inflammatory cytokines like IL-1β
- The lab is interested in investigating the mechanisms of priming and thus, want to transfect the macrophage RAW 264.7 cell line with silencing RNAs to further understand this priming effect
- However, the murine macrophage RAW264.7 cell line is hard to transfect compared to other cell lines including the B16BL6 murine melanoma cell line
- The B16BL6 cell line like the RAW264.7 cell line express functional TLR but lack the PU.1 and CEBPα transcription factors present in macrophages, and these transcription factors are needed for the expression of several inflammatory genes, including the pro-inflammatory cytokine IL-1β[1]
- In contrast, CXCL10 is a pro-inflammatory chemokine that is not PU.1 and CEBPα dependent
- Previous findings showed that PU.1 and CEBPα are myeloid lineage dependent transcription factors for macrophages[1]
- It is currently unknown whether co-transfection and expression of PU.1 and CEBPα in the B16BL6 cell line can give rise to the same priming effects seen in RAW264.7 cells when treated with U0126 prior to LPS exposure

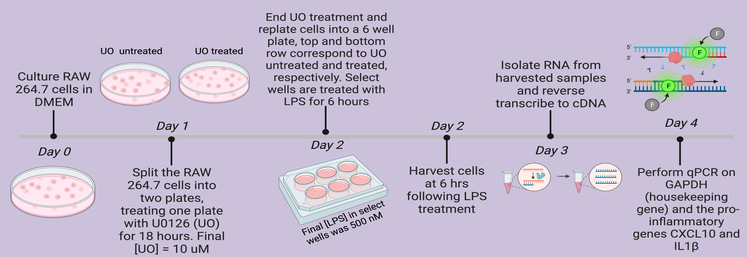
Objective and Hypothesis

Objective: To investigate whether the MEK inhibitor U0126 primes inflammatory responses in B16BL6 cells when co-transfected with PU.1 and CEBPα. This can help inform whether transfected B16BL6 cells may be a viable model for macrophages.

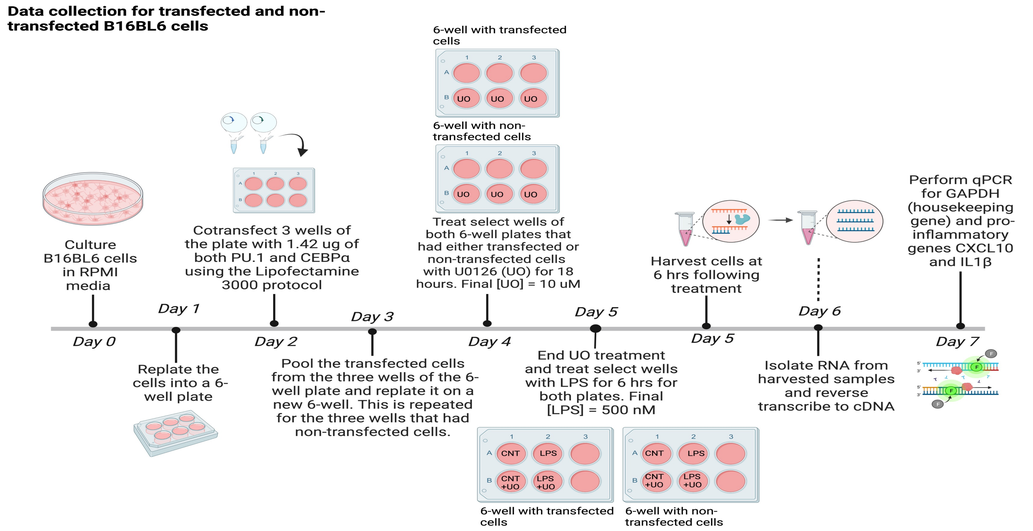
Hypothesis: We hypothesize that transfected B16BL6 cells will exhibit a similar priming effect as the RAW264.7 cells by increasing IL-1β and CXCL10 mRNA expression upon treatment with U0126 prior to subsequent LPS treatment.

Methods

Data collection for the RAW 264.7 macrophage cell line
*This data was collected by the Masters student in the lab



Methods Cont.



Results

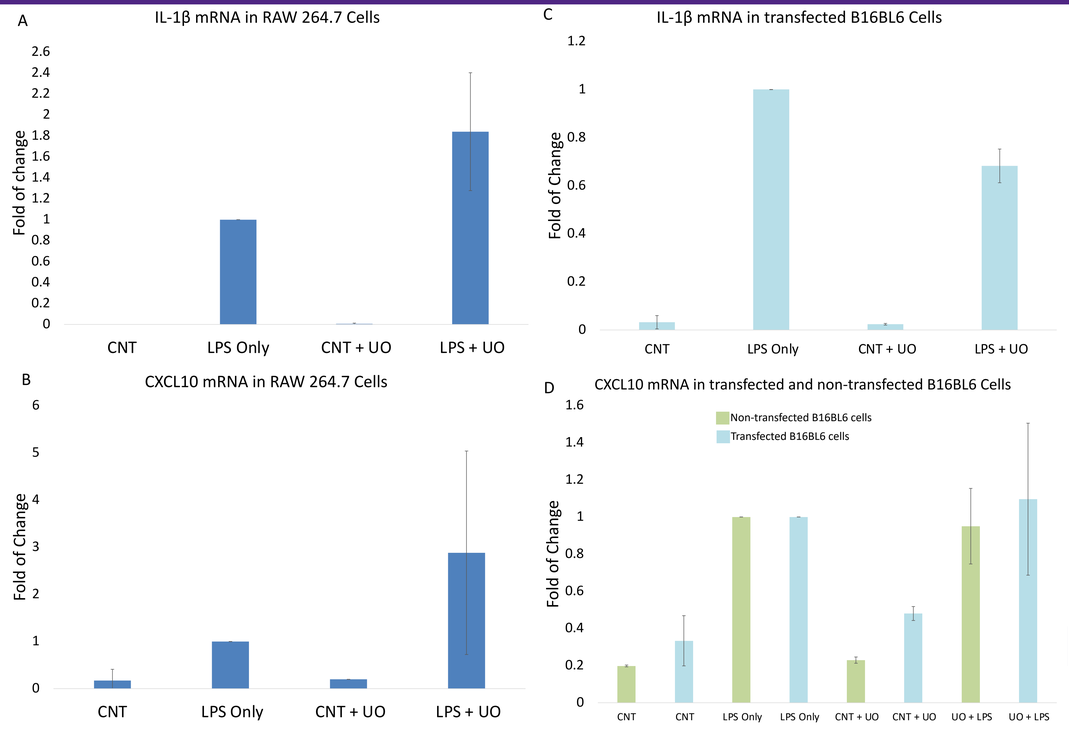


Figure 1. The fold of change of IL-1β and CXCL10 mRNA expression relative to the 6-hour LPS only condition in the murine macrophage RAW 264.7 cells (A, B) and the CEBPα + PU.1 co-transfected murine melanoma B16BL6 cells (C, D). The expression of IL-1β and CXCL10 was determined by RT-qPCR. The fold of change values for all four panels are plotted across four conditions: control, LPS only treatment for 6 hours, control with the 18-hour U0126 (UO) treatment, and the UO 18-hour pretreatment followed by a subsequent 6-hour LPS treatment. The CXCL10 mRNA expression levels in non-transfected B16BL6 cells is also plotted alongside the CXCL10 mRNA expression levels in transfected B16BL6 cells (D). The non-transfected B16BL6 samples serve as the transfection control. The IL-1β mRNA expression levels in non-transfected B16BL6 cells are not plotted as they do not have the PU.1 transcription factor, a component necessary for IL-1β expression. The data are expressed as mean fold of change +/- SD. (A, n = 5; B, n = 3; C and D, n = 2).

Results Cont.

Key Findings:

- In the RAW 264.7 cells, the priming effect is depicted as increased levels of mRNA expression for both CXCL10 and IL-1β in the U0126 pretreatment with subsequent LPS treatment group compared to the expression levels seen in the LPS only group
- For the transfected B16BL6 cells, the mRNA levels of IL-1β appeared to be higher in the LPS only group compared to the U0126 pretreatment followed by the LPS treatment group
    - The CXCL10 mRNA levels appeared to be slightly lower in the LPS only group compared to the U0126 pretreatment followed by LPS treatment group

Discussion and Future Directions

- The results seem to be inconsistent with our hypothesis as the priming effect seen in the RAW 264.7 cells upon pretreatment with U0126 followed by LPS treatment was not observed for IL-1β expression in the transfected B16BL6 cells
- IL-1β mRNA expression is dependent on the presence of PU.1 and since IL-1β was expressed in transfected cells but did not appear to exhibit the priming effect, this might indicate that the priming effect may be more complex and dependent on other factors apart from the PU.1 and CEBPα transcription factors
    - However, more samples need to be collected for the transfected cells to make more conclusive statements
- Although the CXCL10 mRNA expression levels appear slightly higher in the UO pretreatment followed by the LPS treatment group compared to the LPS only group, more samples need to be collected to determine whether there is a clear priming effect
- CXCL10 mRNA expression is known to be PU.1 independent, and thus, it would be expected that the levels of CXCL10 expression in transfected and non-transfected B16BL6 cells would be similar
    - There appears to be slight discrepancy in CXCL10 expression levels in transfected and non-transfected cells in the control, control + UO, and UO + LPS conditions
    - This may be due to experimental technique errors, or alternatively, the PU.1 and CEBPα transcription factors may interact with the transcription factors responsible for CXCL10 transcription in transfected B16BL6 cells, thus giving rise to this observed difference
    - More sample collection can help inform whether these observations are significant
- Our observations point towards a mechanism of priming that may involve other factors beyond PU.1 and CEBPα as well as potential differential priming effects depending on the gene
- Overall, the transfected B16BL6 cells may not be a good model for macrophages as it does not seem to reflect what is seen in RAW 264.7 cells

Future directions

- Upon compiling more samples, it may be interesting to look at the priming effect in transfected cells across other PU.1 independent and dependent genes to see if results are consistent or if they vary
- It may also be worthwhile to look at using a different model that is more like macrophages

References and Acknowledgements

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References

1. Ha, Soon-Duck et al. “The transcription factor PU.1 mediates enhancer-promoter looping that is required for IL-1β eRNA and mRNA transcription in mouse melanoma and macrophage cell lines.” *The Journal of biological chemistry* vol. 294,46 (2019): 17487-17500.

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