Cell Differentiation: The Case of Turning THP-1 Cells into M1 Macrophages

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Introduction

The Calin lab focuses on the role of miRNAs in cancers and works to find new biomarkers and therapeutic targets. MiRNAs are small non-coding RNAs that regulate gene expression. THP-1 is a cell line of monocytes, a type of white blood cell, from an acute myeloid leukemia patient. **Aim:** The goal of this project is to differentiate these cells into M1 macrophages, which are a type of macrophages that inhibit tumor growth, and characterize the macrophages based on their markers and the miRNAs they express.

Methods

THP-1 cells were cultured and differentiated into M0 macrophages with phorbol 12-myristate 13-acetate (PMA). After differentiation, cells were activated into M1 macrophages using lipopolysaccharide (LPS) at concentrations of 1, 10, and 20 ug/mL for 6 and 24 hours. Before the addition of LPS, an MTS assay, in which MTS reacts with viable cells to form a brown-colored product, was done to test the cell viability after treating with LPS.

Total RNA was extracted from treated and control cells and the concentration was analyzed using Nanodrop. Total RNA was reverse transcribed into cDNA and RT-qPCR was used to analyze the expression level of markers (IL 1β, IL 12α, and IFN γ)[1] and miRNAs (miRNA 125A, miRNA 146A, and miRNA 155)[2] associated with M1 macrophages induced with LPS [Figure 1]

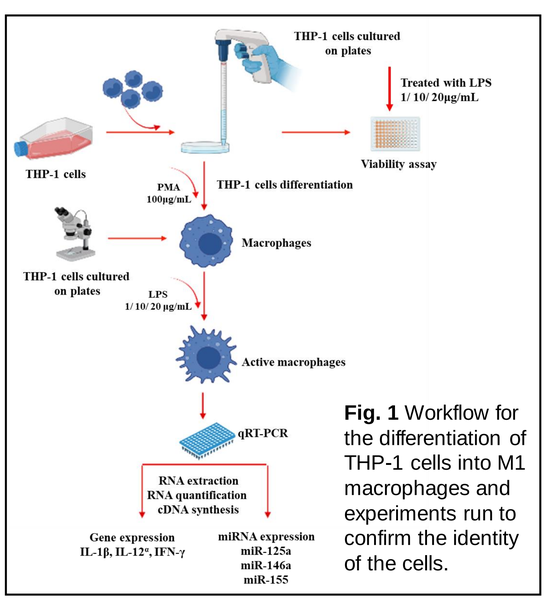

Fig. 1 Workflow for the differentiation of THP-1 cells into M1 macrophages and experiments run to confirm the identity of the cells.

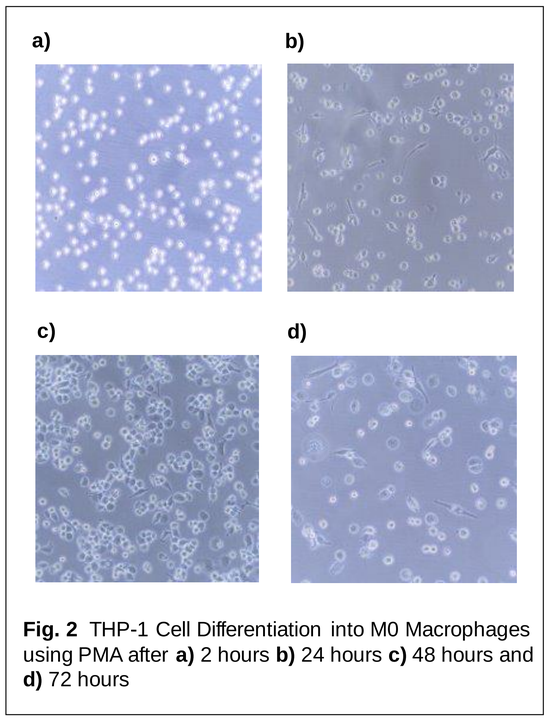

Fig. 2 THP-1 Cell Differentiation into M0 Macrophages using PMA after **a)** 2 hours **b)** 24 hours **c)** 48 hours and **d)** 72 hours

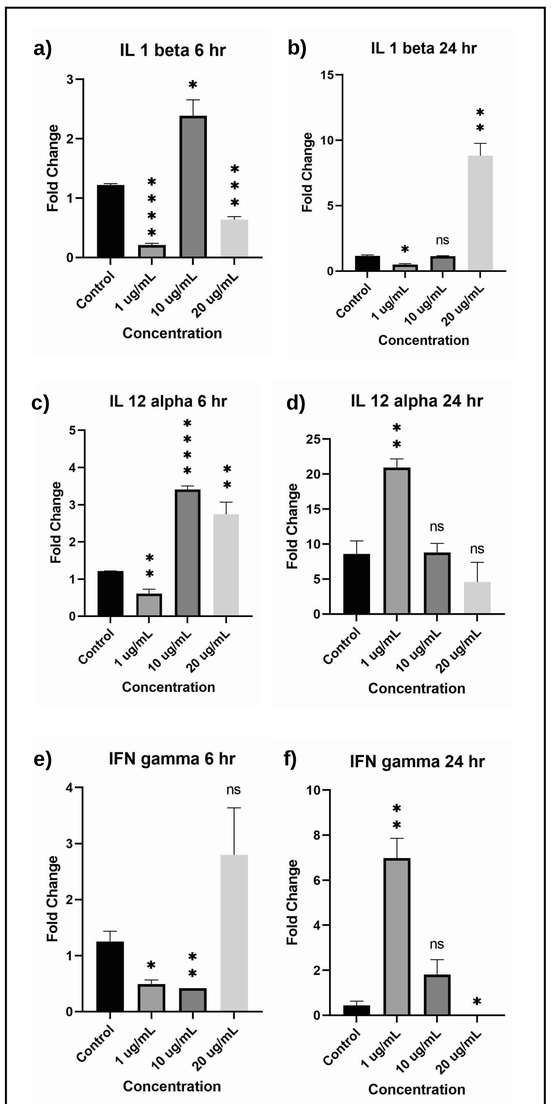

Fig. 3 RT-qPCR results testing the change in the gene expression of various markers associated with M1 macrophages after 6 hours and 24 hours of treatment with LPS. **a)** change in IL-1 beta after 6 hours **b)** change in IL-1 beta after 24 hours **c)** change in IL 12 alpha after 6 hours **d)** change in IL 12 alpha after 24 hours **e)** change in IFN gamma after 6 hours **f)** change in IFN gamma after 24 hours. T-test was performed as a statistical analysis. ns is a p value greater than or equal to .05. * is a p value <.05, ** is a p value < .01, *** is a p value < .001, and **** is a p value of < .0001.

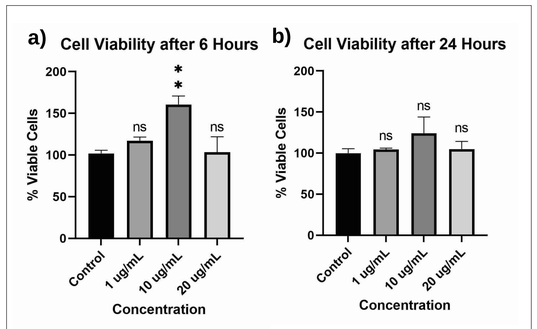

Fig. 4 THP-1 Cell Viability after treating with LPS and tested using an MTS Assay. **a)** after 6 hours of treatment **b)** after 24 hours of treatment. T-test was used to perform the statistical analysis. ** represents a p value of < 0.01.

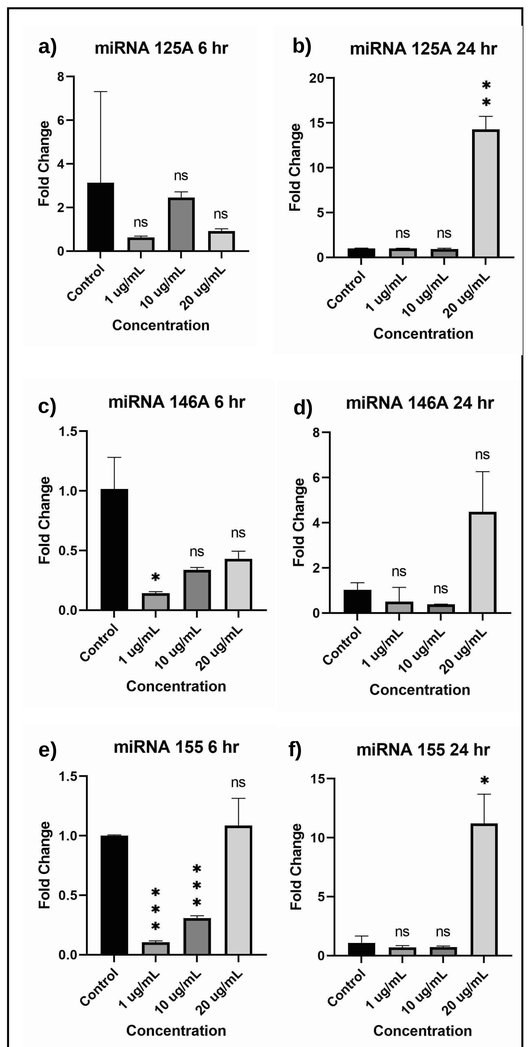

Fig. 5 RT-qPCR results testing the change in expression level of various miRNA associated with M1 macrophages differentiated with LPS after 6 hours and 24 hours of treatment. **a)** change in miRNA 125A after 6 hours **b)** change in miRNA 125A after 24 hours **c)** change in miRNA 146A after 6 hours **d)** change in miRNA 146A after 24 hours **e)** change in miRNA 155 after 6 hours **f)** change in miRNA 155 after 24 hours. T-test was used to perform a statistical analysis. ns is a p value greater than or equal to .05. * is a p value <.05, ** is a p value < .01, *** is a p value < .001, and **** is a p value of < .0001.

References

1) Cháves-Galán, L. Olleros, M.L., Vesin, D., & Garcia, I. (2015). Much more than M1 and M2 macrophages, there are also CD169+ and TCR+ macrophages. *Frontiers in Immunology.* https://doi.org/10.3389/fimmu.2015.00263

2) Graff, J.W., Dickson, A.M, Clay, G., McCaffrey, A.P., & Wilson, M.E. (2012). Identifying Functional MicroRNAs in Macrophages with Polarized Phenotypes. *Journal of Biological Chemistry, 287*(26), 21826-21865. https://doi.org/10.1074/jbc.M111.327031

Results

After differentiating with PMA into M0 macrophages, the cells attached after 2 hours and changed morphology after 72 hours [Figure 2]. The MTS Assay showed that the cell viability was not affected by treating cells with LPS [Figure 4].

With 1 ug/mL LPS for 6 hours, the cells showed a decrease expression level in all the genes and miRNAs. With 10 ug/mL LPS for 6 hours, the cells showed an increased expression level in IL 1β and IL 12α and decreased expression level in IFN γ and all miRNAs. With 20 ug/mL LPS for 6 hours, the cells showed an increased expression level in IL 12α, IFN γ, miRNA 125A, and miRNA 146A and a decreased expression level in IL 1β and miRNA 155 [Figures 3 and 5].

With 1 ug/mL LPS for 24 hours, the cells showed an increased expression level in IL 12α and IFN γ, a decreased expression level of IL 1β, miRNA 146A, and miRNA 155, and no change in expression level in miRNA 125A. With 10 ug/mL LPS for 24 hours, the cells showed an increased expression level in IFN γ, a decreased expression level in miRNA 146A, and no change in the other genes and miRNAs. With 20 ug/mL for 24 hours, the cells showed an increased expression level in IL 1β, a decreased expression in the other genes and all of the miRNAs [Figures 3 and 5].

Conclusion

I concluded that the cells differentiated into M0. The MTS assay shows that the LPS does not affect the viability of the cells.

For the macrophage genes, I concluded that the 10 ug/mL for 6 hours and 1 ug/mL for 24 hours treatments worked best. This means that the activation into M1 macrophages is both time and dose dependent. The miRNA expression is also time and dose dependent as only the 20 ug/mL LPS for 24 hours showed increased expression of the miRNAs.

Acknowledgements

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