Characterisation of the monocyte-like THP1 cell line response to hypoxia pathway activation *in vitro*

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INTRODUCTION

Monocytes play key roles in human health and disease, with their importance in human lung fibrosis having recently been identified [1]. However, this remains poorly characterised, with some evidence to suggest that this may be a consequence of hypoxia inducible factor (HIF) pathway activation [2].

THP-1 is a human leukaemia monocytic cell line which has been extensively used to study monocytes *in vitro*. Importantly, there are both similarities and differences between these immortalized cells and primary human peripheral blood monocytes, which require careful characterisation. To investigate the role of monocytes in lung fibrosis, we selected THP-1 cells as a stable and reproducible cell line with which to model monocyte behaviour, comparing cell morphology and cell surface marker expression of THP-1 cells to human primary monocytes. Furthermore, we have investigated the THP-1 response to HIF pathway activation to improve our understanding of how this may contribute to fibrotic processes.

AIM

We aim to characterise THP-1 cells and their response to hypoxia.

METHODS

- THP-1 cells were obtained from The European Collection of Authenticated Cell Cultures (ECACC) (THP-1 ECACC 88081201). Human primary monocytes were isolated from donor blood.
- A morphological characterisation of THP-1 cells and primary monocytes was performed on cell cytospin stained with Romanowsky-Giemsa using light microscopy, with CD14 cell surface marker expression on THP-1 cells assessed by cytofluorimetry.
- THP-1 cells were incubated under hypoxic (1% oxygen tension) and normoxic conditions for 24 hours. Additionally, cells were treated with various doses of the hypoxia mimicking compound, dimethyloxalylglycine (DMOG), for 24 hours under normoxic conditions. We then investigated changes in specific gene, protein and metabolite markers of hypoxia.
- We assessed the metabolic consequences of HIF pathway activation in DMOG-treated THP-1 cells using the real time Seahorse XFp T Cell Metabolic Profiling Kit.

THP-1 cells are morphologically similar to human primary monocytes and express the surface marker CD14

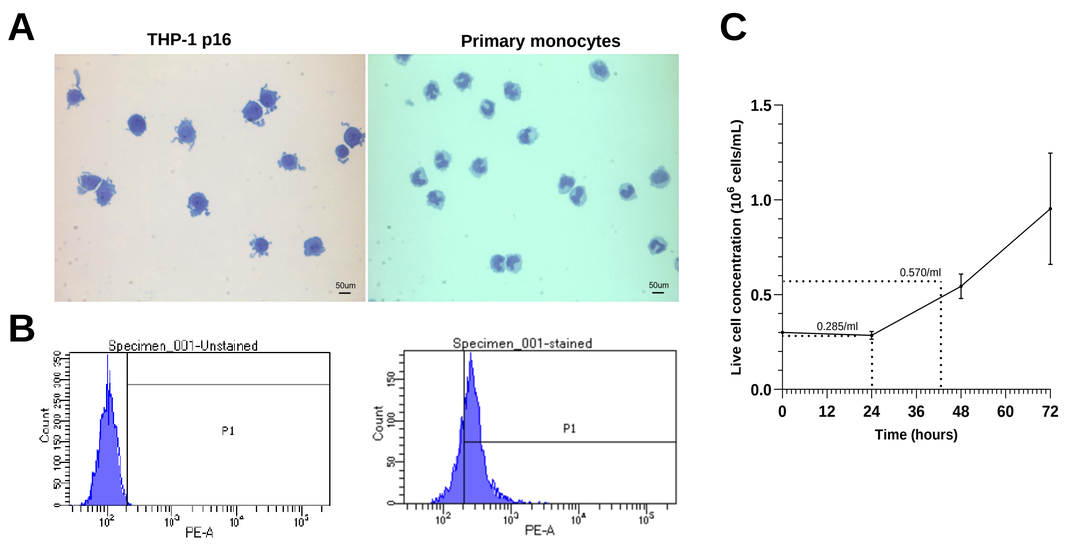

Figure 1: Characterisation of THP-1 morphology, CD14 expression and growth rate. (A) Light microscopy images of THP-1 and human primary monocyte cells at 400X. Scale bar included for reference. **(B)** FACS analysis of THP-1 CD14 marker expression. Cells were stained with anti-CD14 PE-labelled antibodies, with ~70% of cells positively staining for CD14 expression. **(C)** Growth curve of THP-1 cell expansion obtained from 3 independent cell expansions. Dotted lines represent the expected doubling time, extrapolated from the live cell concentration at 24 hours. Error bars represent the mean ±SD (n=3).

Hypoxia and hypoxia mimetic treatment stabilises HIF-1α protein expression in THP-1 cells

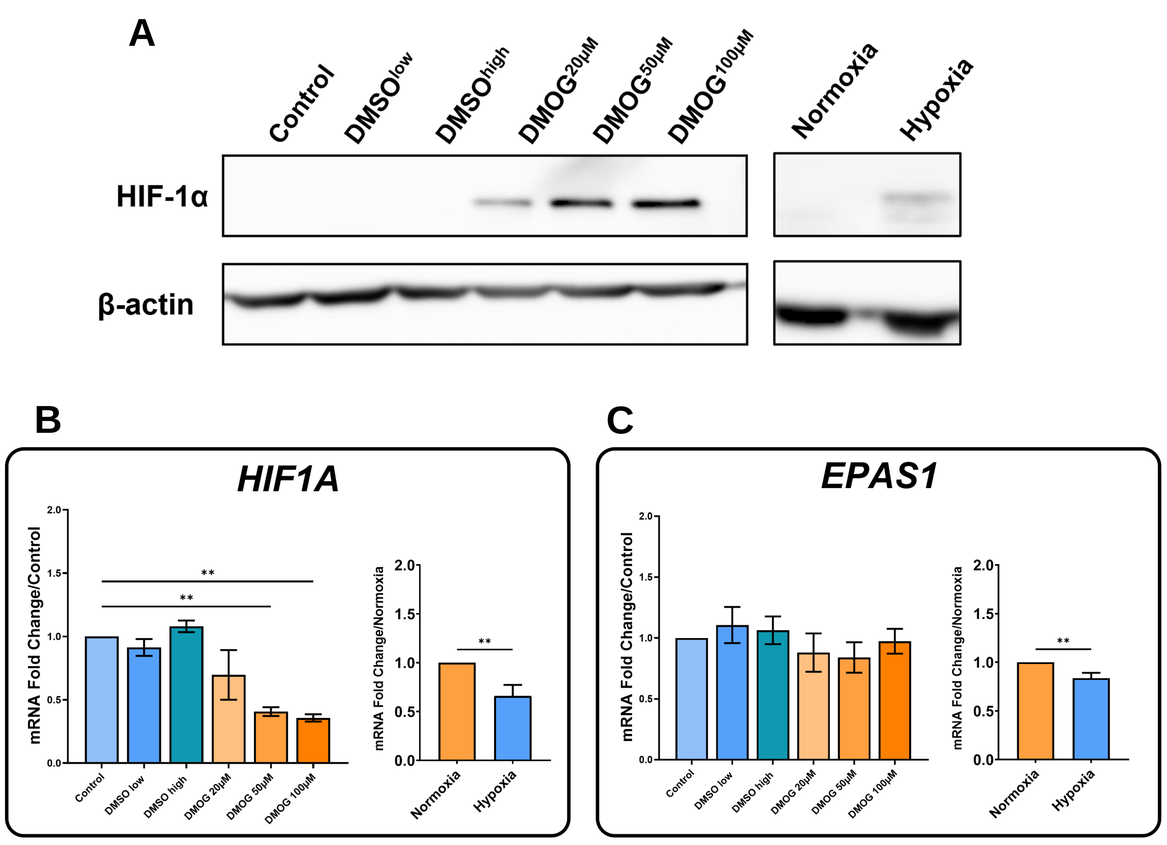

Figure 2: HIF protein and gene expression in THP-1 cells treated for 24 hours with DMOG versus DMSO-matched vehicle controls and in THP-1 cells incubated for 24 hours under normoxic versus hypoxic conditions. Data was obtained from three independent experiments. (A) Western blots for HIF-1α, with a protein band detected at ~132kD. β-actin was used as the loading control. **(B-C)** RT-qPCR analysis of genes associated with the HIF pathway; *HIF1A* **(B)** and *EPAS1* **(C)**. Relative gene expression was calculated using the ΔΔCT method, with bars displaying the mean ±SD (n=3 for each gene). Statistical analysis was performed using a one-way ANOVA with post-hoc Dunnett's multiple comparison test for DMOG comparisons, with a Student's t-test performed for normoxia versus hypoxia comparisons; **p<0.01.

HIF pathway activation induces the expression of genes in THP-1 cells associated with a hypoxic and monocytic immune cell response

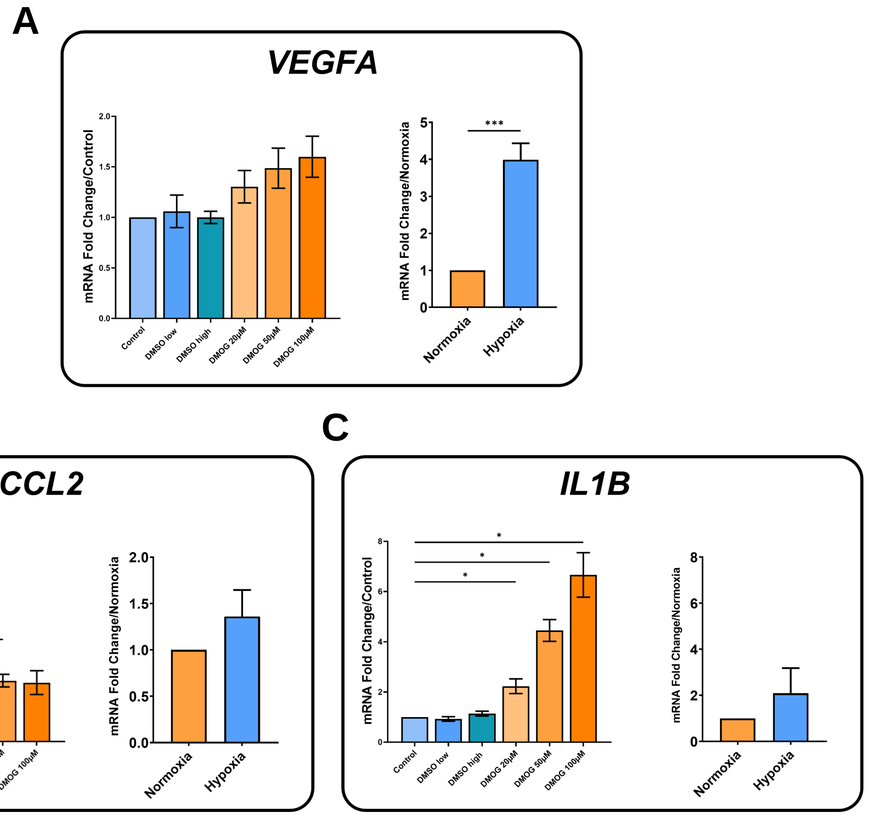

Figure 3: Gene expression changes for genes regulated by HIF pathway activity in THP-1 cells treated for 24 hours with DMOG versus DMSO-matched vehicle controls and in THP-1 cells incubated for 24 hours under normoxic versus hypoxic conditions. Data was obtained from three independent experiments. Relative gene expression was calculated using the ΔΔCT method, with bars displaying the mean ±SD (n=3 for each gene). Statistical analysis was performed using a one-way ANOVA with post-hoc Dunnett's multiple comparison test for DMOG comparisons, with a Student's t-test performed for normoxia versus hypoxia comparisons; *p<0.05, ***p<0.001.

HIF pathway activation promotes a metabolic switch from aerobic to glycolytic ATP production

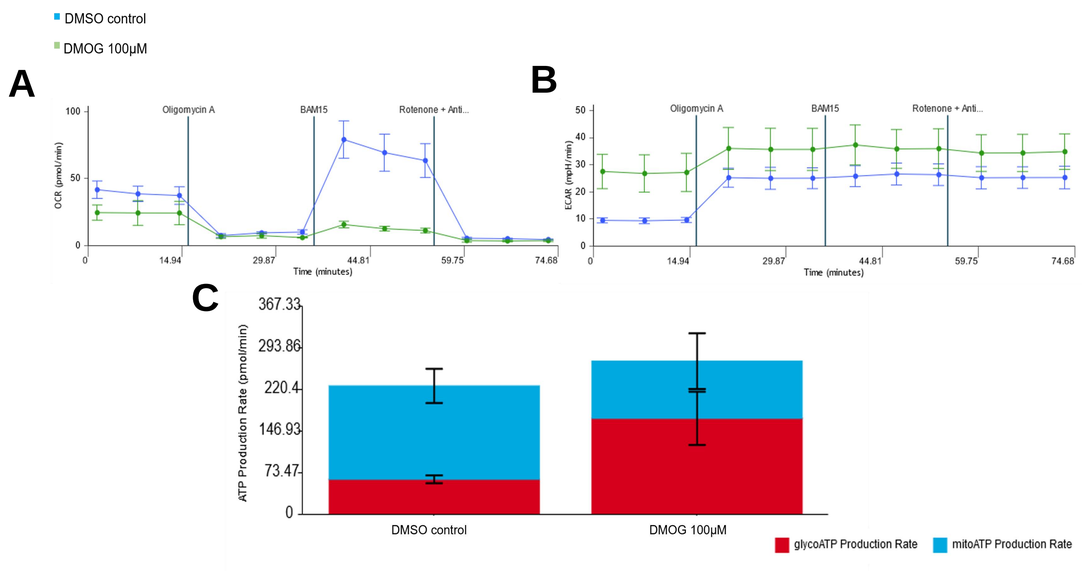

Figure 4: THP-1 cells undergo a metabolic switch from aerobic to glycolytic ATP production in response to HIF pathway activation. (A-B) Oxygen consumption rate (OCR) **(A)** and extracellular acidification rate (ECAR) **(B)** of THP-1 cells treated with either 100μM of DMOG (green line) or a DMSO-matched vehicle control (blue line). Bars represent the mean ±SD (3 replicate wells per condition). **(C)** The ATP Production Rate was calculated using OCR and ECAR values obtained in response to DMOG and the DMSO-matched vehicle control. Bars represent the mean ±SD (3 replicate wells per condition). **(D)** Lactate concentration quantified obtained from THP-1 supernatants in cells incubated under normoxia or hypoxia. Error bars representing the mean ± SD (n=3), with a Student's t-test performed for normoxia versus hypoxia comparisons; *p<0.05.

DISCUSSION & CONCLUSIONS

- THP-1 cells are morphologically similar to human primary monocytes and express the canonical monocyte marker, CD14.
- Hypoxic stress in THP-1 cells induces HIF-1α stabilization and subsequent HIF pathway activation, as supported by the upregulation of genes associated with both the cellular hypoxic response and the monocytic immune response.
- Hypoxic stress also promotes a metabolic switch from aerobic to glycolytic ATP production in THP-1 cells.
- These cellular responses are similar to that observed in *ex vivo* human primary monocytes and therefore support the use of THP-1 cells as a stable, reproducible "off-the-shelf" reagent for human disease relevant studies of HIF pathway stabilization.

1. Scott MKD, Quinn K, Li Q, Carroll R, Warsinske H, Vallania F, et al. Increased monocyte count as a cellular biomarker for poor outcomes in fibrotic diseases: a retrospective, multicentre cohort study. Lancet Respiratory Medicine. 2019;7(6):497-508.
2. Zhou Y, Ewing RM, Davies DE, Wang Y, Jones MG. A validated hypoxia-inducible factor (HIF) signature across tissue compartments predicts clinical outcome in human lung fibrosis. medRxiv. 2022:2022.01.11.22269085.