Paper Presentation: Metabolic regulation of gene expression by histone lactylation

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Epigenetics, Gene Organization & Expression
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Presentation Overview

- Background
- Summary of paper and methods
  - Discovery of lactylation
  - Histone lactylation and glycolysis
  - Stimulation of lactylation
  - Lactylation gene expression profile
  - Lactylation mechanism of action
  - Conclusion
- Critique
- Future Experiment

Background

- Lactate is formed from pyruvate metabolism
- M0 macrophages are activated to polarize into M1 or M2 phenotypes
- Warburg effect → a tumor induced microenvironmental shift characterized by hypoxia, angiogenesis, macrophage polarization, and T cell activation

Discovery of lactylation

- HPLC and MS/MS showed a lactyl group on histone lysine residues in human MCF-7 cells
- Synthetic lactylated histone lysines developed to confirm identity
  - Pan anti-Kla antibody and isotopic heavy lactate used for further confirmation
- MS data showed other lactylation sites in HeLa cells and mouse bone marrow-derived cells

Discovery of lactylation - Data

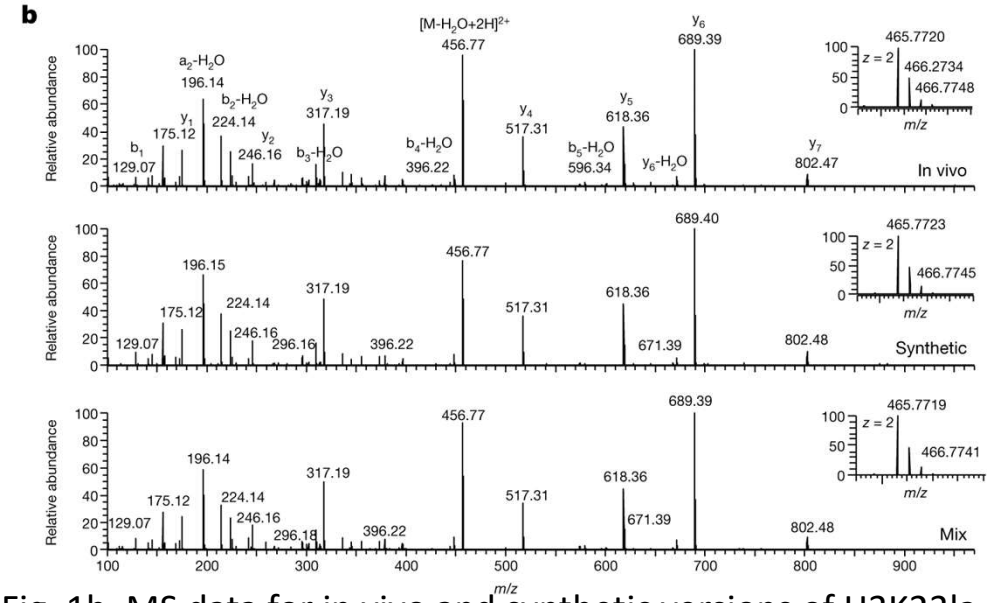


Fig. 1b. MS data for in vivo and synthetic versions of H3K23la shows that they are equivalent.

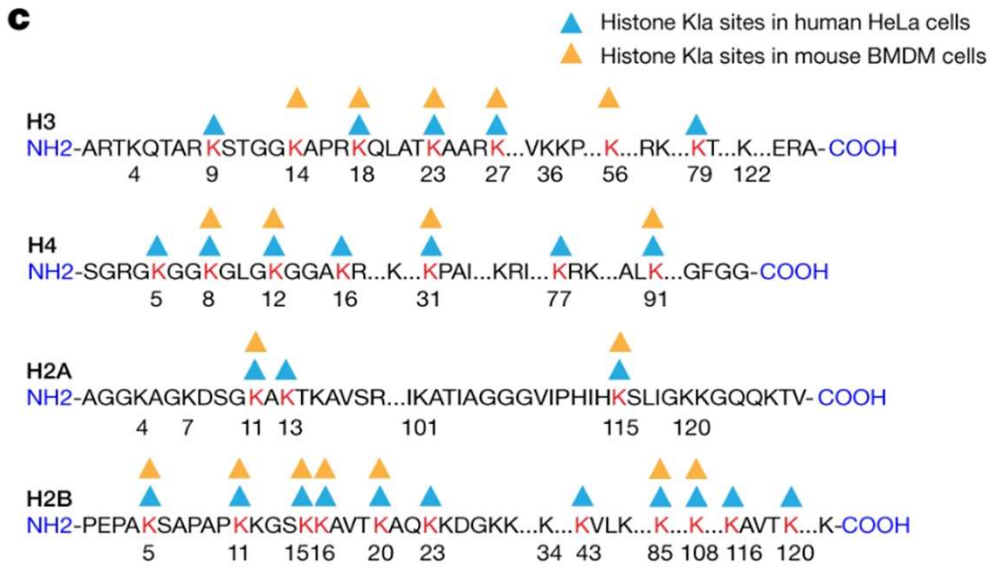


Fig. 1c. Histone lactylation sites discovered by MS

Histone lactylation and glycolysis

- Glucose addition increases lactate concentration and lactylation levels
- Non-metabolizable glucose analogue (DG) decreased lactate and lactylation levels
- Lactate metabolic pathway modification can increase or decrease lactylation
- Isotopic glucose and MS/MS confirm the lactate is metabolically derived
- Rates of glucose utilization differ between acetylation and lactylation

Histone lactylation and glycolysis - Data

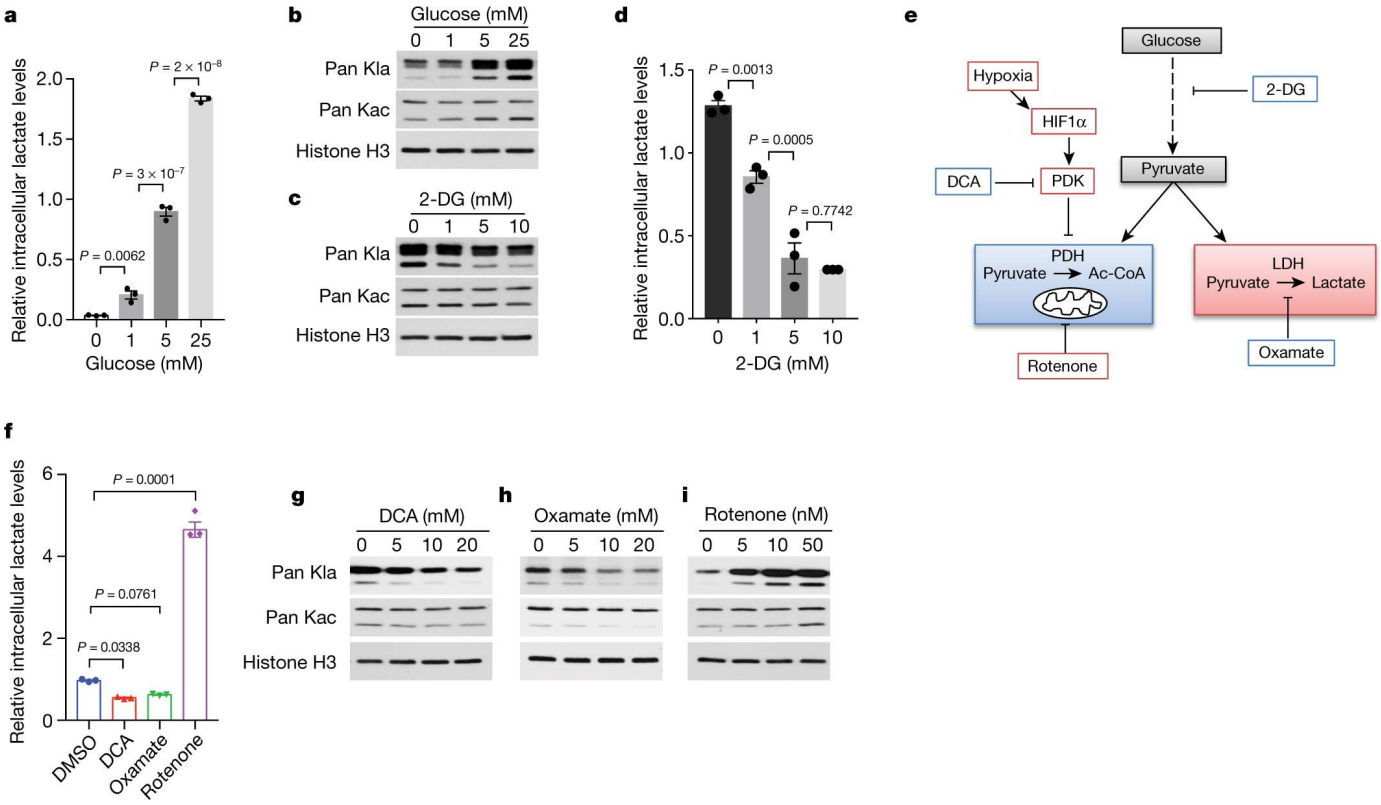


Fig 2a-i. Increased glucose increases lactate and lactylation in MCF-7 cells

Stimulation of lactylation - Hypoxia

Hypoxia

- Caused increased lactate and lactylation levels in MCF-7 cells
  - Confirmed by radiolabeling
  - Results also seen in HeLa and RAW 264.7 cells
- Hypoxia-induced lactate production can be inhibited by oxamate, DCA, and LDHA/LDHB deletion
  - LDHA/LDHB deletion was completed in HepG2 cells, but they were not otherwise used due to poor viability

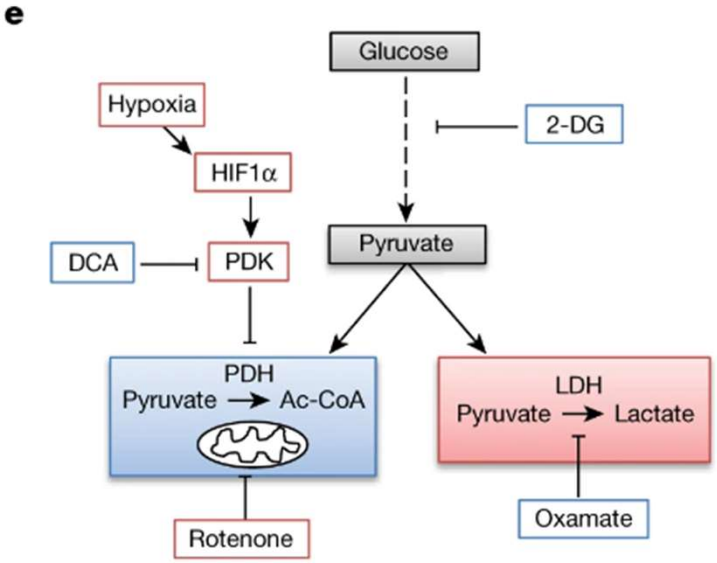


Fig 2e. Glucose metabolic pathway and modulators

Stimulation of lactylation – Hypoxia Data

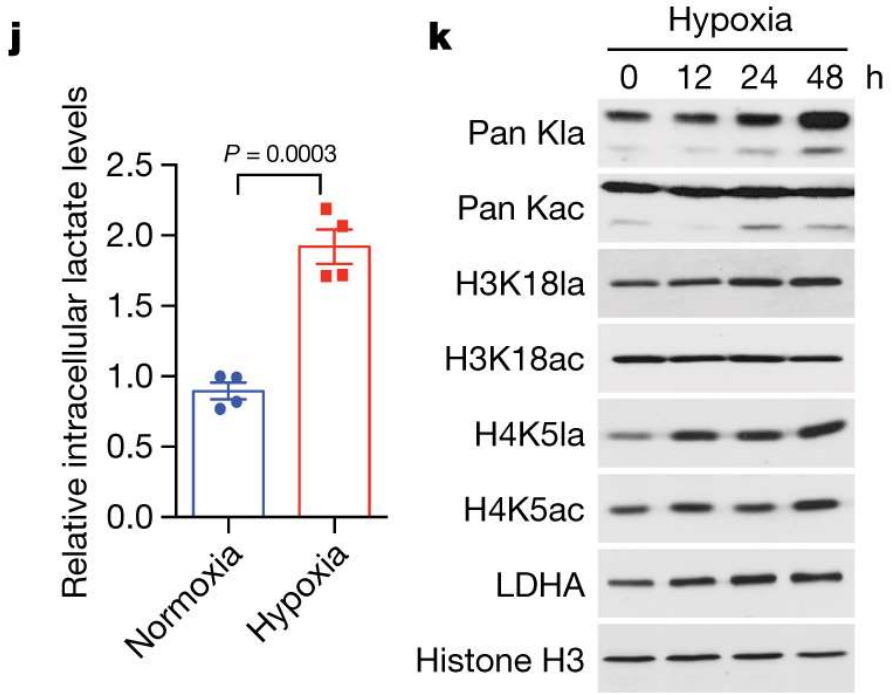


Fig 2j, k. Hypoxia stimulates lactate production and lactylation in MCF-7 cells

Stimulation of lactylation - Immunity

Immunity

- Macrophages polarized to M1 phenotype using LPS and IFNγ
- Lactate and lactylation levels increased after 16-24 hours
  - Confirmed by radiolabeling using glucose
- Media changed every 4 hours to rule out signaling effect between cells
- These changes did not occur in M2 macrophages

Stimulation of lactylation - Data

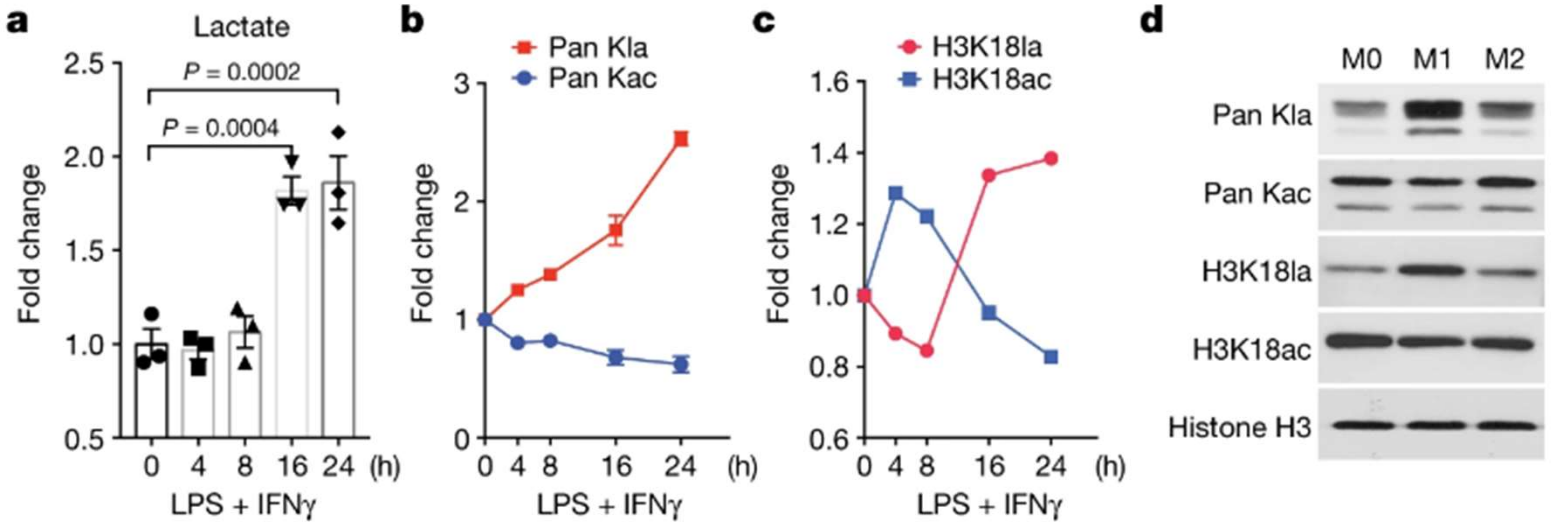


Fig. 3a-d. M1 polarization induces lactate production and lactylation

Lactylation gene expression profile 1 of 2

- 24 hours after M1 polarization, macrophage gene expression analyzed using RNA-seq, ChIP-seq, ChIP-qPCR, and immunoblotting
  - Anti-H3K18la and anti-H3K18ac antibodies used
- Transition from M0 to M1 caused an increase in lactylated histones at promoters
  - 68% of genes marked by H3K18la were specific to H3K18la
- Inflammatory gene activation differed temporally from H3K18la marked genes
  - GO analysis indicated H3K18la specific genes are mostly inflammation independent and there is some M2-like expression of Arg1
- Confirmatory testing with various bacteria as immune stimuli produced similar results

Lactylation gene expression profile 1 of 2 - Data

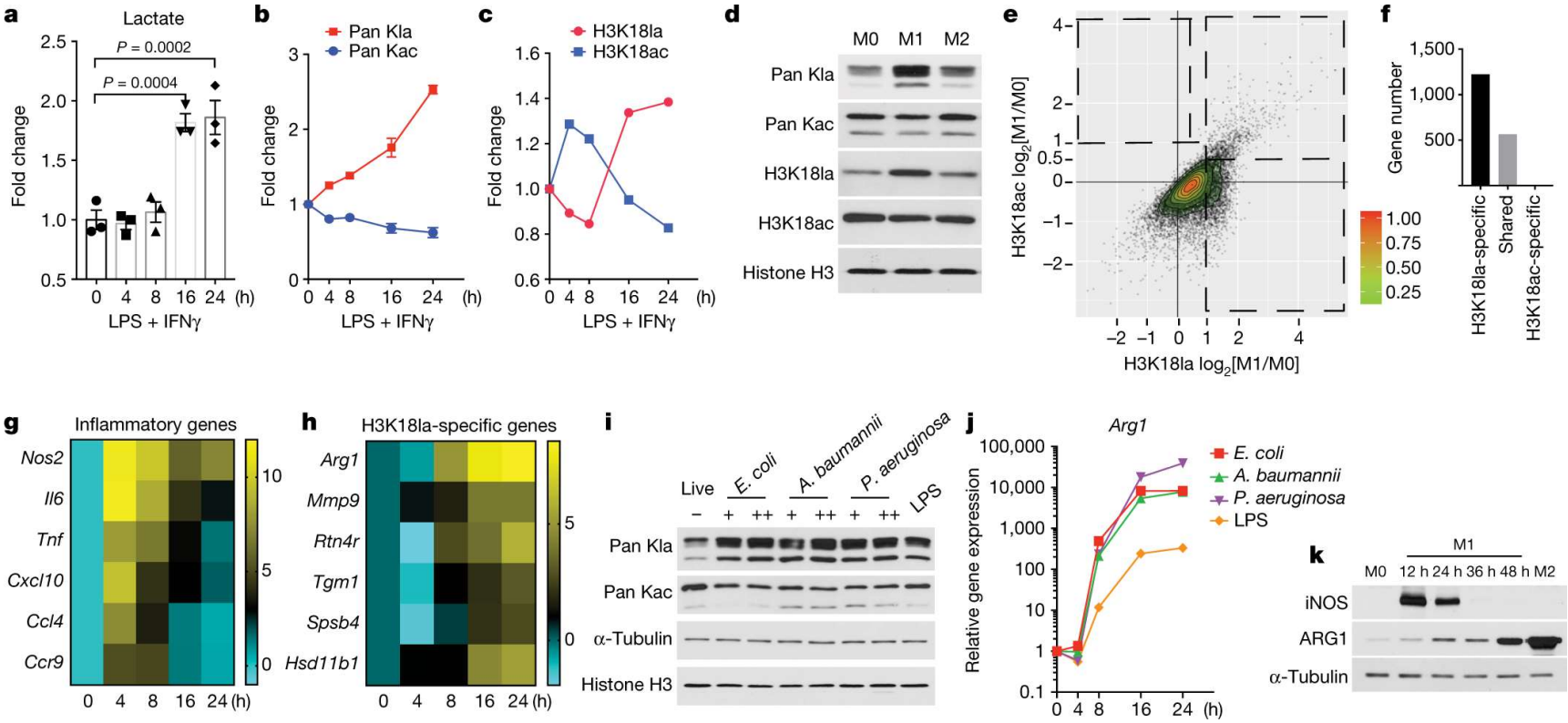


Fig. 3e-k. H3K18la is associated with increased gene expression that is independent of pro-inflammatory genes

Lactylation gene expression profile 2 of 2

Arg1 expression was examined under:

- Lower lactate concentration
  - LDHA gene was deleted
  - Intracellular lactate, Arg1 expression, and H3K18la marks at the Arg1 promoter decreased
  - Inflammatory gene expression was unchanged
- Higher lactate concentration
  - Lactate added to media
  - Intracellular lactate, Arg1 expression, and H3K18la increased
  - Inflammatory gene expression was unchanged
  - Expression of other M2-like genes increased as well

Lactylation gene expression profile 2 of 2 - Data

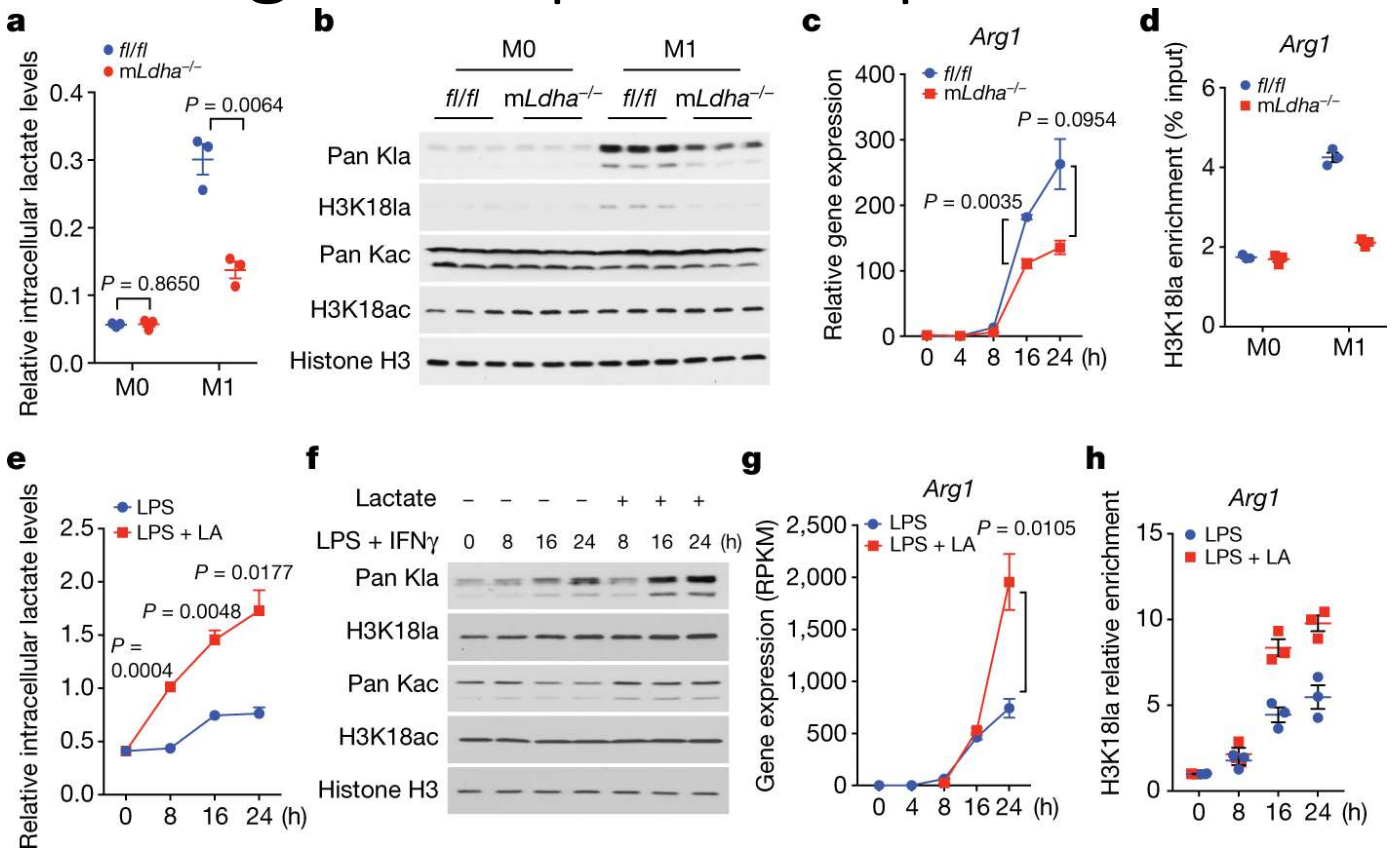
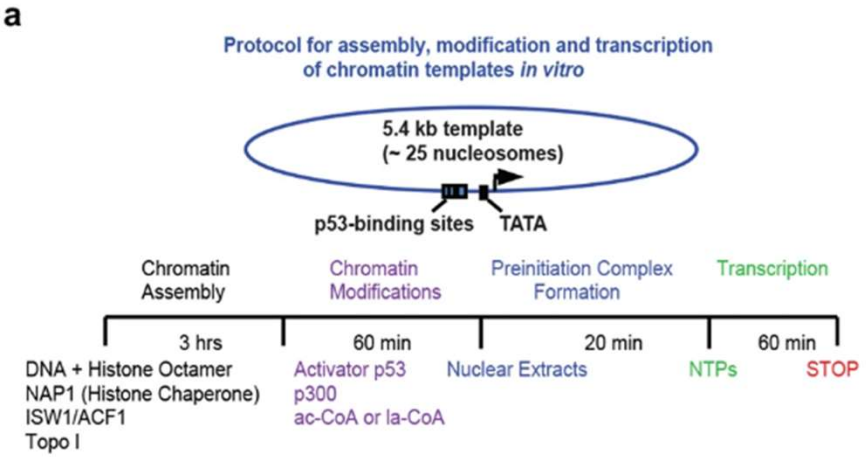


Fig. 4a-h. Increased lactate concentration on Arg1 expression

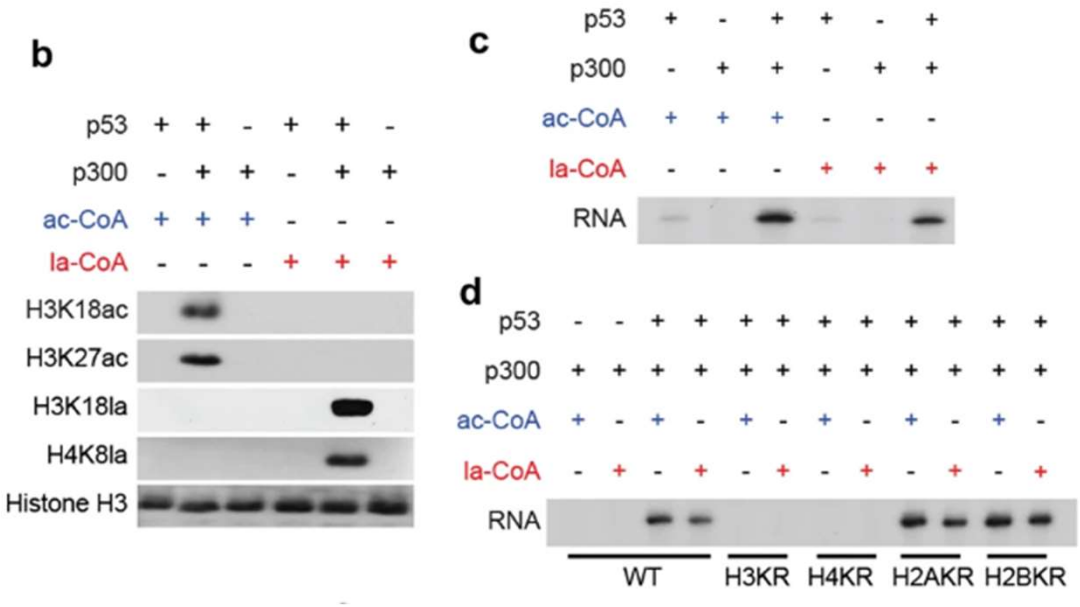
Lactylation mechanism of action

- Confirms histone lactylation as a direct mediator of transcription
- Cell-free, recombinant chromatin-templated histone modification and transcription assay used
  - This is used to confirm the relationship between lactylation and gene expression
- Results show p53- and p300-dependent histone Kla directly activates transcription, similar to Kac



Extended Data Fig. 9a. Simplified assay protocol

Lactylation mechanism of action - Data

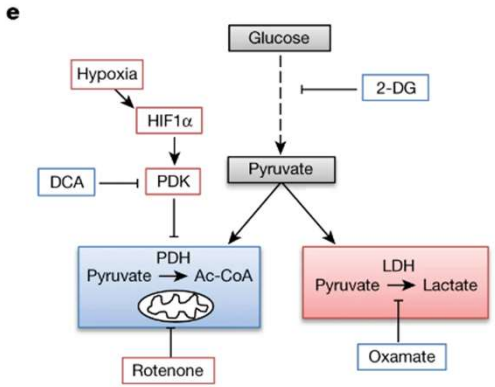


Extended Data Fig. 9b-d. Cell-free chromatin and transcription assay results. P300 mediated, p53 induced lactylation activates transcription

Conclusion

- Lactate production during M1 polarization acts as a molecular clock for the switch to a M2-like expression profile
  - This switch is carried out epigenetically by histone lactylation
- Histone lactylation does not induce or repress inflammatory genes
- The Kla modification is a fundamental mechanism that will likely reveal more about cancer pathophysiology

Critique



Histone lactylation and glycolysis:

- Extraneous testing of metabolic inhibitors

Hypoxia stimulus:

- Overlap with DCA testing, partially justified by other cells lines sharing results
- HepG2 results could be excluded

Immune stimulus:

- GNE-140 testing could be excluded

Lactylation mechanism of action:

- Extended data fig. 9a-c should be figures in the main paper
- Extended data fig. 9e-g were extraneous experiments

General:

- Included the necessary information in the main paper, most confirmatory and extra information is in the extended data
- Well supported findings through confirmatory radiolabeling, multiple replicates, and immunoblotting
- Conclusions are appropriate for the information they obtained

Future Experiment

Subject: Lactylation as an epigenetic control mechanism in skeletal muscles

Methods:

- Pilot experiment in vitro:

  Quantify presence of histone lactylation in mouse skeletal muscle cells

  - Conditions: Normal media, lactate addition, hypoxic conditions
  - Methods: HPLC, MS/MS, Immunoblotting, radiolabeling

- Secondary experiment in vivo:

  Quantify histone lactylation levels in muscle cells from two groups of mice (normal activity vs. increased activity) to investigate lactylation's effect on skeletal muscles

  - Methods: HPLC, MS/MS, Immunoblotting, radiolabeling, RNA-seq, CHIP-seq

References

Zhang, D., Tang, Z., Huang, H. *et al.* (2019). Metabolic regulation of gene expression by histone lactylation. *Nature* **574**, 575–580. https://doi.org/10.1038/s41586-019-1678-1