Investigating the effect of chronic inflammation on mitochondrial function in a prematurely ageing mouse model

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Introduction

There are undisputed links between chronic inflammation and ageing. In this project, a mouse model of chronic low-level inflammation has been utilised to see if there are any links between chronic inflammation and mitochondrial dysfunction. This mouse lacks expression of the p50 subunit of NF-κB, which in its homodimer form (p50:p50) functions as an important epigenetic repressor of inflammatory gene expression. This results in accelerated ageing of the mouse and a lack of tissue regeneration.

Aims

- Analyse the effect of chronic low level inflammation in nfkb1-/- mice on mitochondrial respiratory chain protein expression using immunofluorescence techniques.
- Measure levels of protein expression using immunofluorescence microscopy.
- Use statistical techniques to test the difference between complex I, complex IV and porin expression at a significant level.

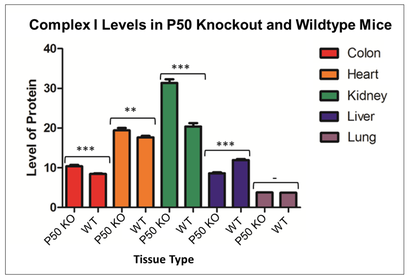
Methods

The same antibodies were used for each tissue during the immunofluorescence staining. These were reached after optimization of the technique:

- Primary Antibodies:
  - NDUFB8 IgG1
  - MTCO1 IgG2a
  - VDAC1 IgG2b
- Secondary antibodies were conjugated to the fluorophores FITC, TRITC and CY5.
- These identified the levels of Complex I, Complex IV and Porin, respectively.
- Fluorescence intensity for both wildtype and p50 knockout mice for each tissue type were quantified using imageJ.
- Unpaired T-test was used to measure the significance of the results. The p50 knockout and wildtype mouse data was divided into groups and compared.
- Graphs showing the average and standard deviation of each group were produced to show if there was a positive or negative change in protein expression.

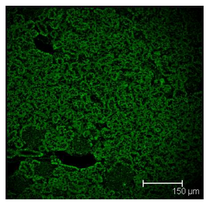
Results – Complex I

Complex I (NADH dehydrogenase) is a key component of the mitochondrial respiratory chain reaction. It is one of the main sources of the premature leak of electrons to oxygen, leading to the creation of superoxide's which can damage the mitochondria. This graph shows the differences in complex I expression in mouse tissue, where p<0.05 = *, p<0.01 = **, p<0.001 = ***:

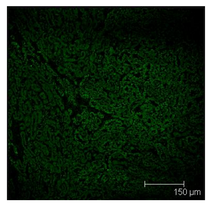


The difference between Complex I expression in P50 and wildtype mice can be most easily observed visually in kidney tissue:

P50 knockout Mouse 8 20X

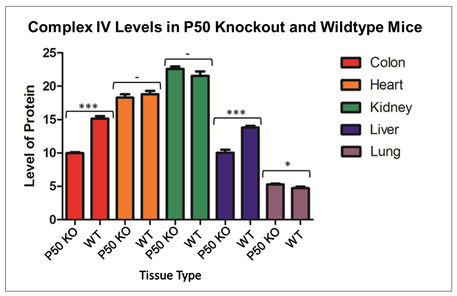


Wildtype Mouse 1 20X



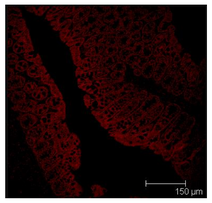
Results – Complex IV

Complex IV (cytochrome c oxidase) contributes to the proton gradient in the mitochondrial respiratory chain. This graph shows the differences in complex IV expression in mouse tissue:

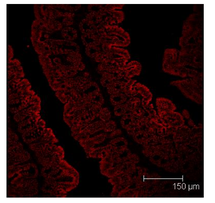


The difference between Complex IV expression in P50 and wildtype mice can be most easily observed visually in colon tissue:

P50 knockout Mouse 3 20X

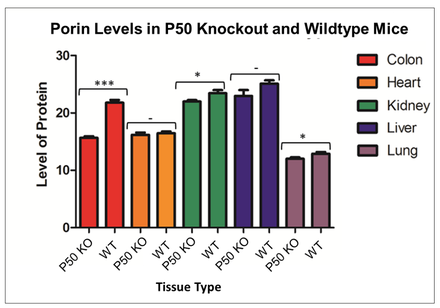


Wildtype Mouse 1 20X



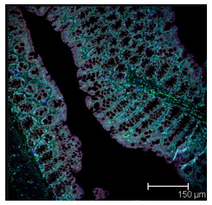
Results – Porin

Porins are a group of integral proteins in the outer membrane of mitochondria. They were used in this study to detect if the p50 gene affects mitochondrial mass. This graph shows the differences in porin expression in mouse tissue:

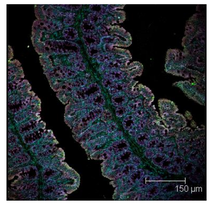


Porin was stained blue which helped to show co-localisation of porin with complex I and complex IV. The cyan areas show co-localisation between complex I and porin and the purple areas show co-localisation between complex IV and porin in this colon tissue:

P50 Knockout Mouse 3 20X



Wildtype Mouse 1 20X



Conclusions

- P50 knock out mice have altered expression of essential subunits of the respiratory chain in a tissue specific manner.
- Mitochondrial density is up-regulated in all tissues studied.
- This effect may be due to increased reactive oxygen species production in the p50 knock out animals due to chronic inflammation or cellular senescence.
- Further research could investigate the expression of reactive oxygen species markers or senescent cells alongside the mitochondrial proteins studied here to explore these possible links.

References

- Escames, Germaine, et al. "Mitochondrial DNA and inflammatory diseases." Human genetics 131.2 (2012): 161-173.
- Mariappan, Nithya, et al. "NF-κB-induced oxidative stress contributes to mitochondrial and cardiac dysfunction in type II diabetes." Cardiovascular research 85.3 (2010): 473-483.
- Cogswell, Patricia C., et al. "NF-κB and IκBα Are Found in the Mitochondria EVIDENCE FOR REGULATION OF MITOCHONDRIAL GENE EXPRESSION BY NF-κB." Journal of Biological Chemistry 278.5 (2003): 2963-2968.