Inhibition of mitochondrial protein synthesis with doxycycline induces biosynthesis of OXPHOS Complex V

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Background

Mitochondria are structures in the cell. Their main function is the production of ATP – a vital energy carrier (Alberts et al., 2013). Mitochondria make ATP through 5 enzyme complexes during a process called oxidative phosphorylation (OXPHOS). Mitochondria have evolved from bacteria. Therefore, some antibiotics that kill bacteria also affect mitochondrial function.

Doxycycline is an antibiotic that inhibits protein synthesis in bacteria as well as mitochondria. (Patel & Parmar, 2020). However, contrary to expectations, the host lab found that doxycycline induces biosynthesis of 2 mitochondrial proteins, MTATP6 and MTATP8, while protein synthesis of all other mitochondrial proteins is reduced (unpublished data, host lab). Both MTATP6 and MTATP8 are part of OXPHOS Complex V, also known as ATP Synthase, which is the terminal protein complex responsible for production for ATP (Ahmad et al., 2020).

Understanding the effect doxycycline has on Complex V may potentially grant scientists a treatment or diagnosis option for mitochondrial diseases, which are currently untreatable and easily misdiagnosed.

Method

A549 lung carcinoma cell cultures were used as cell model. Cells were treated with doxycycline for up to 5 days, followed by harvesting and extraction of the proteins from the sample.

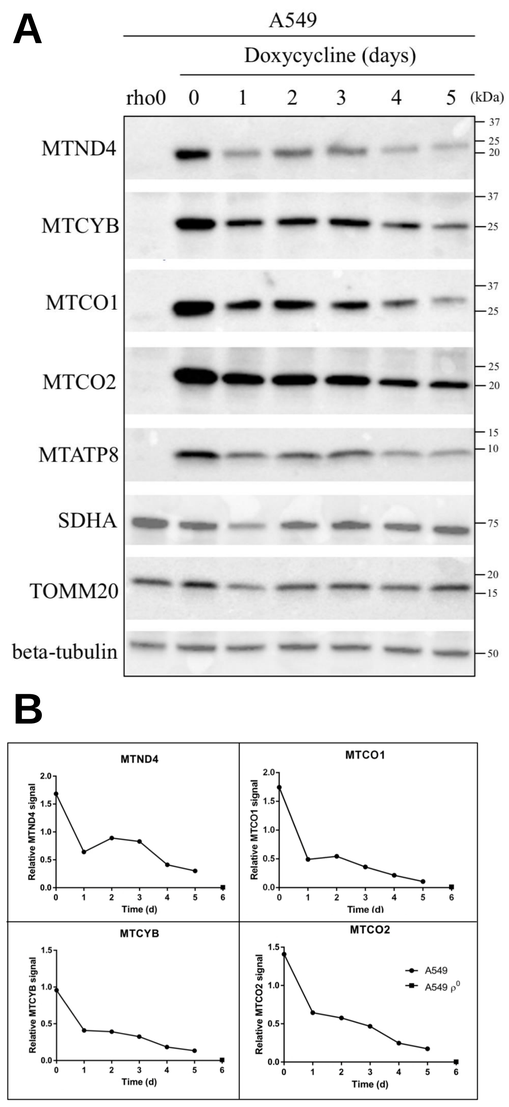
To accurately make up samples, the protein concentrations were determined.

Separate proteins were analysed on denaturing gels and whole proteins were analysed using native gels.

Using native gels, I also measured enzyme activity of complexes V and II.

A: Western blots of separate proteins using denaturing gels.
B: Graphs with relative protein signal and days of doxycycline treatment
C: Western blots of whole OXPHOS Complexes using native gels.
D: In-Gel Enzyme activity stain
E: Graph with relative ATP5A protein signal and days of doxycycline treatment

Results

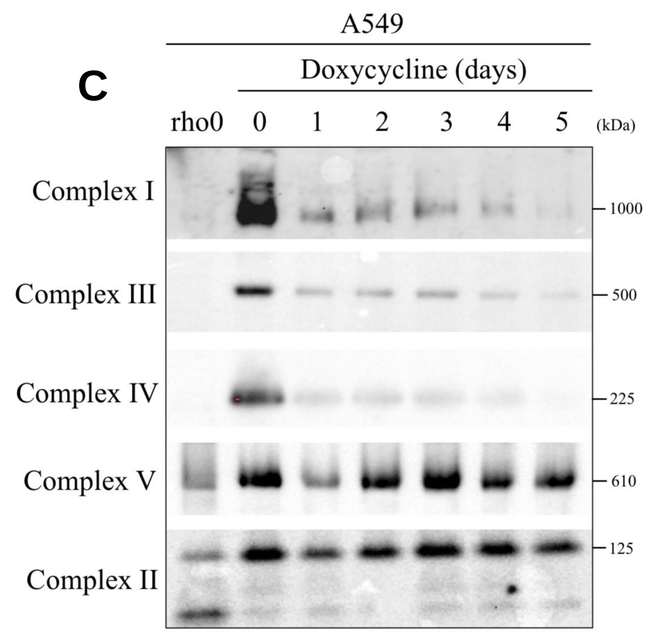


When probing the blots of denaturing gels with SDHA antibodies, we found the 1 day sample to have a lower concentration of mitochondrial proteins reflected by a lighter band in Figure A.

Taking this into account, as expected the concentration of proteins MTND4, MTCYB, MTCO1 and MTCO1 all steadily decrease with doxycycline treatment. This is evident in the graphs of corrected band intensity to SDHA concentration showing a steady decline (Figure B).

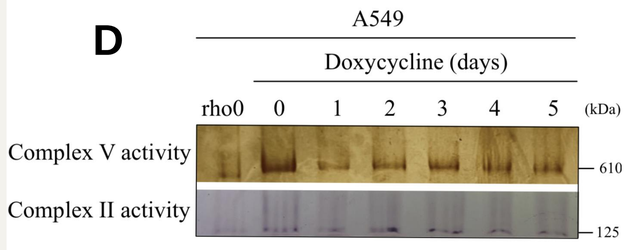
MTATP8 concentration is less affected after prolonged doxycycline treatment (days 2 and 3).

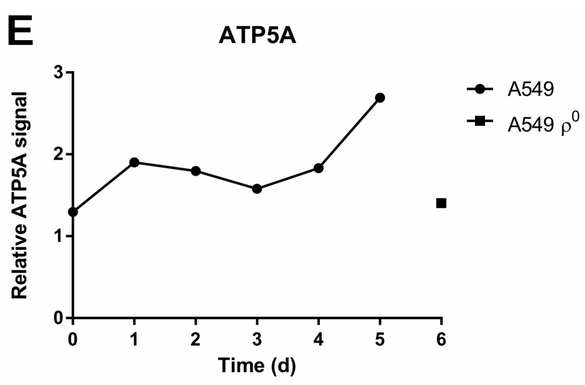
Results



MTND4 is found in complex I, MTCYB in Complex III, MTCO1 and MTCO2 in Complex IV, while MTATP8 in Complex V.

Results from native gels reveal the same pattern of the concentration of Complexes I, III and IV going down, while Complex V remains the same.





In-gel enzyme activity of Complex II decreases, while remaining the same for Complex V. This is seen in the graph of ATP5A which is a protein found in Complex V, ATP Synthase.

The main finding of this project is that the inhibition of mitochondrial protein synthesis by doxycycline appears to preserve biosynthesis of OXPHOS Complex V, while biosynthesis the other complexes is decreased.

More in-depth research in this field may potentially lead to understanding the pathological mechanism of mitochondrial diseases better as well as the purpose of this compensatory mechanism.

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