CHIEF SCIENTIST OFFICE

CODE: CAF/21/13

RESEARCH PROJECT BRIEFING

Impaired ketogenesis mediates intestinal stem cell mitochondrial function in Ulcerative Colitis (UC)

BACKGROUND

Ulcerative colitis (UC) is a common condition affecting the large bowel where the bowel lining becomes very inflamed. Flare-ups of UC can severely impact a patient's quality of life. Key to replenishment of the intestine are intestinal stem cells (ISCs). These cells are very active and consume large amounts of energy. Cells generate energy from mitochondria or ***'cell batteries'***.

We hypothesise that the mitochondria, are damaged in UC intestinal stem cells, leading to the large intestine to have ***'faulty batteries'***, which causes impaired healing observed in UC.

KEY FINDINGS

- The mitochondrial or ***'cell batteries'*** are damaged in UC ISCs
- Damaged mitochondria within UC ISCs lead to propagation of ***'faulty batteries'*** through the entire gut lining.
- Concurrently, there is also loss of a ***'back up energy supply'*** through loss of fasting energy supply (ketogenesis) in UC
- We find that upon restoration of fasting pathways, we can repair stem cell function.
- We provide the first combined evidence of mitochondrial dysfunction and ketogenesis failure ***'primary'*** and ***'backup' energy failure*** at the ISC level in UC. Crucially, restoring ketogenesis reverses ISC pathogenic defect thus, opening a new 'immuno-metabolic' therapeutic approach in UC.

WHAT DID THE STUDY INVOLVE?

We developed techniques that facilitated growth of patient derived organoids or ***'mini guts'*** from patients that have not received medical treatment before. This facilitates study of human intestinal stem cell function.

To determine the role of mitochondrial dysfunction in UC we used the following advanced scientific techniques on epithelial cells and patient derived organoids:

- Single cell RNA sequencing (scRNA-seq).
- Single cell quantitative immunofluorescence.
- Single cell energetic metabolism by profiling translation inhibition (SCENITH).

The Edinburgh Inflammatory Bowel Disease Science group led by Dr Ho has its own integrated Patient Public group that is intimately involved with our research (led by Edinburgh patients, K McGuire and J Rysdale) and working very closely with patient charities, Crohn's Colitis UK and Guts UK charity.

We have also worked closely with out PPI group to facilitate:

- The lay summary of this PhD proposal.
- Design of study protocol, patient information sheet and lay summaries for the Edinburgh Gut Research group large studies (MARVEL – www.marvelstudy.uk, MUSIC – www.musicstudy.uk and GI-DAMPs)

We have directly created and produced a short film 'Our lives with IBD' detailing the scientific work of our group and its direct translation to medical therapy for patients, which was premiered at Edinburgh Science Festival in 2024. Reviewed here: https://www.thelancet.com/journals/langas/article/PIIS2468-1253(24)00128-6/abstract.

WHAT WERE THE RESULTS AND WHAT DO THEY MEAN?

Firstly, we developed organoid culture allowing isolation and culture of organoids or ***'mini guts'*** from human large bowel biopsies obtained at endoscopy **(Fig. 1)**.

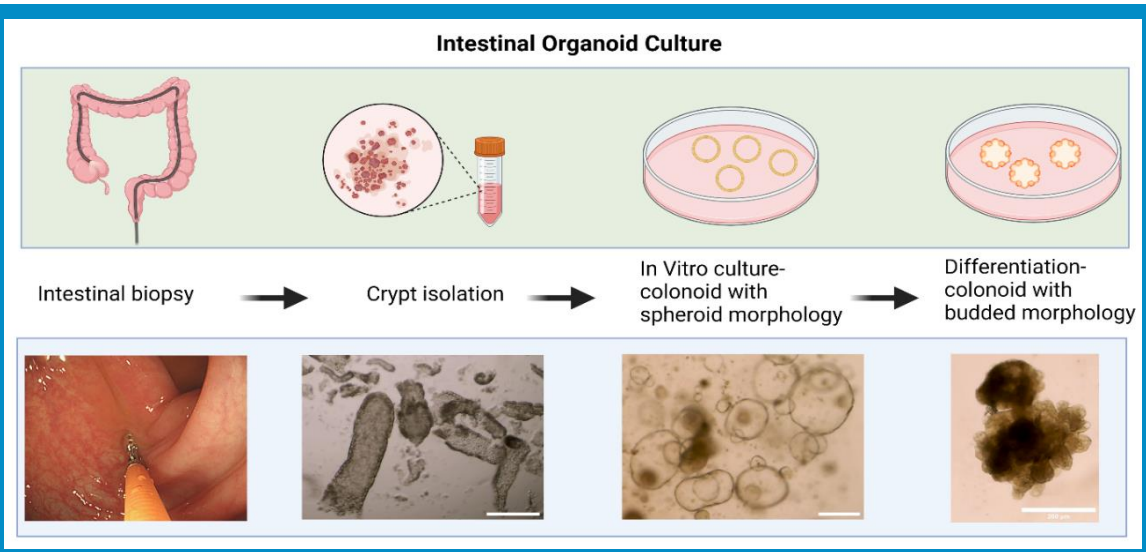


Fig. 1: Culture of ISCs as organoids from intestinal biopsies.
Displaying tissue sampling (left), large bowel lining isolation (middle left), organoid culture (middle right), budded organoid(right). Scale bar 250µm. Adapted from (Rutherford and Ho, 2023)

Result 1. Mitochondrial function is impaired in UC stem cells

We observed significant reductions energy production proteins or ***'faulty batteries'*** in samples taken from the lining of the large intestine in UC patients

In UC ***'mini guts'***, we find that ***'faulty batteries'*** are passed from ISCs to occupy the entire lining of the large bowel **(Fig. 2).**

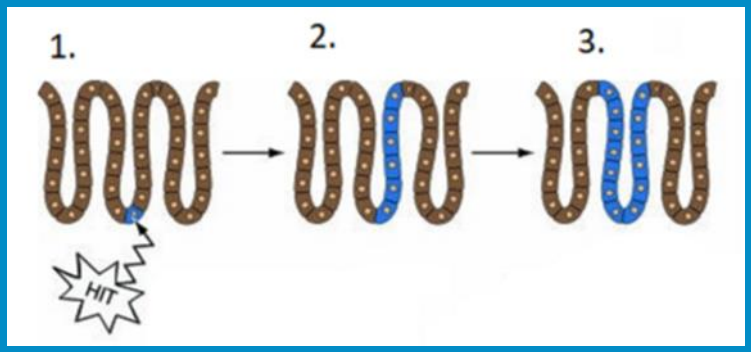


Fig. 2: Expansion of mitochondrial mutations from crypt base through clonal expansion

WHAT WERE THE RESULTS AND WHAT DO THEY MEAN?

Result 2. Failure of backup energy supply in UC intestinal stem cells

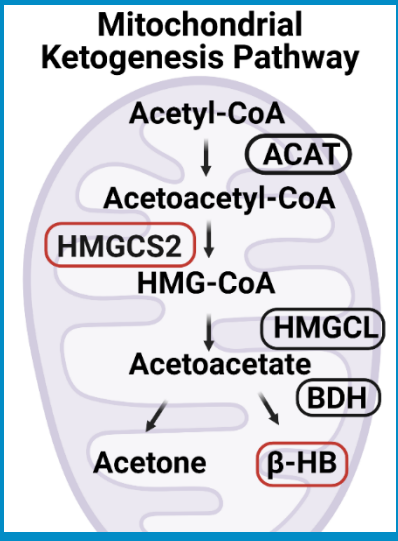
We also find that pathways that regulate fasting pathways, typically switched on in humans in response to low energy conditions to produce ketones are abnormally switched off in UC stem cells ***'failure of backup energy supply'***. Crucially, these fasting pathways also occur in the mitochondria, and so the mitochondrial damage we have found in UC may cause less ketones to be produced.

Result 3. Restoration of fasting pathways fixes cell batteries and intestinal stem cells

We find that we can improve stem cell function by administering drugs that act on pathways to reverse damage to fasting pathways. Moreover, when we administer ketones to ***'mini guts'***, we find that we can improve mitochondrial function and reduce cellular stress, which also leads to improved stem cell function. Finally, we see that in patients that are able to respond well to medical therapy and heal the lining of their intestines, fasting pathways are active, suggesting that this is important mechanism in UC.

Fig. 3 Mitochondrial ketogenesis cascade

Ketogenesis is the process where fatty acids are broken down in the mitochondria to produce ketone bodies. HGMCS2 is the rate limiting enzyme in the cascade. The main product, **β-hydroxybutyrate (BHB)**, serves as an alternative energy source, especially during fasting or low carbohydrate intake, providing energy when glucose is scarce.



WHAT IMPACT COULD THE FINDINGS HAVE?

- Together, our findings suggest that impaired fasting pathways leads to reduced healing of the lining of the intestine in the UC. This may open possibilities for new treatments to increase the fasting signal in UC.
- Future work would involve commencement of a randomised control trial investigating the role of drugs that activate fasting pathways for treatment of UC.

HOW WILL THE OUTCOMES BE DISSEMINATED?

This work has been presented at the following international conferences:
- Keystone mitochondrial dysfunction, Colorado, USA 2023
- DDW 2024, Washington DC
- Keystone immunometabolism 2024, Kerry Ireland
- GlasgowGastro GSG, Awarded first prize poster presentation, Glasgow 2024

The next phase of dissemination will involve publication of our work in a high impact open access journal.

CONCLUSION

We provide the first combined evidence of mitochondrial dysfunction and ketogenesis failure ***('primary' and 'backup' energy failure***) at the stem cell level in UC. Crucially, restoring ketogenesis reverses ISC pathogenic defect thus, opening a new 'immuno-metabolic' therapeutic approach in UC.

RESEARCH TEAM & CONTACT

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Additional Information

Project completion August 2024