CHIEF SCIENTIST OFFICE

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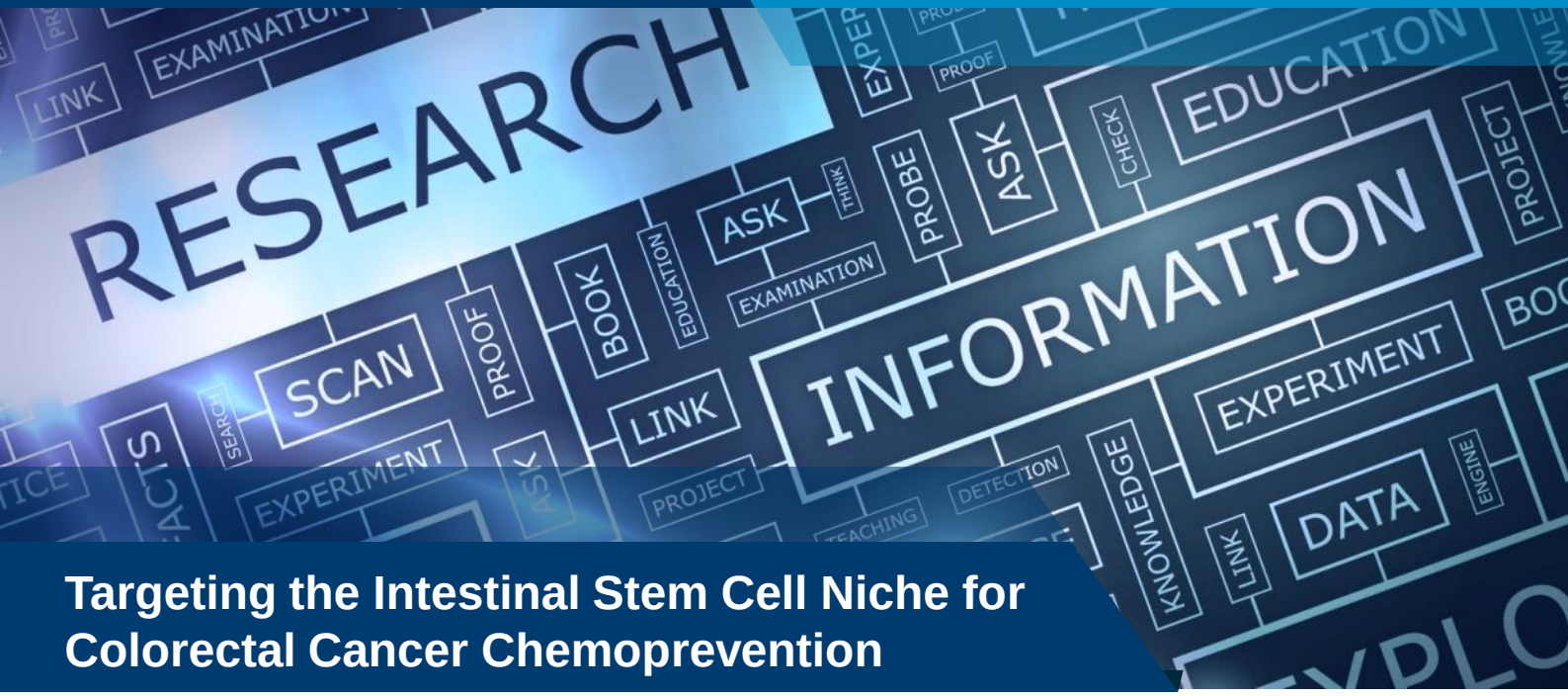
RESEARCH PROJECT BRIEFING




# Targeting the Intestinal Stem Cell Niche for Colorectal Cancer Chemoprevention

## AIMS

Bowel cancer, the second leading cause of cancer deaths in the UK, has a considerable preventable component (~54%). Risk factors include diabetes, obesity, and metabolic syndrome and highlight the strong link between metabolism, diet and bowel cancer. Given the national increase in obesity, we need to understand the ways in which nutrient (food) overload can lead to bowel cancer. We think that excessive nutrients lead to bowel cancer by increasing certain types of cells called stem cells. Cells communicate with each other through signals that tell a cell how to behave. We know that common drugs like aspirin and metformin decrease the risk of getting bowel cancer and may decrease stem cells. Hence, there is powerful rationale to study how known bowel cancer preventive agents may reset abnormal metabolism and stem cells by affecting specific molecular signalling pathways in cells.

Our Scottish population data show that aspirin will not prevent bowel cancer in all people taking these drugs and so it is important to identify who will respond so we can get the 'right drug' to the 'right patient'. Metformin is also emerging as a powerful chemopreventive agent and raises the question of whether both agents could target abnormal bowel cells in a more effective way. Here, we aimed:

- to identify how aspirin may decrease the cancer stem cell population in bowels cancer and discover potential biomarkers for response
- to understand whether nutrient overload with fat leads to an increase in cancer stem cells and which signalling pathways are targeted to rescue this increase in cancer stem cells.
- to identify signalling pathways that are only affected when both aspirin and metformin are given at the same time targets which may shed light on shared targets that could be exploited for chemoprevention in patients.

## KEY FINDINGS

- Aspirin can reverse the Wnt-driven cystic phenotype in human organoids whilst reducing stem cell marker expression and increasing expression of Dickkopf-1, a Wnt antagonist often lost during CRC progression
- Aspirin decreases specific pathways involved in sensing nutritional cues (lipid signalling)
- Novel signalling targets identified in when bowel cells exposed to both aspirin and metformin compared to either drug alone

## WHAT DID THE STUDY INVOLVE?

The research project used different techniques in the laboratory to study various aspects of bowel cancer. They focused on studying RNA (genetic material), proteins, and properties of stem cells. They conducted these studies using both bowel cancer cell lines and 3D structures called 'mini-guts' grown from patients with bowel cancer or genetic conditions that increase the risk of bowel cancer, like familial adenomatous polyposis (FAP).

In simpler terms, they looked at genetic information, proteins, and features of stem cells in both cancer cells and mini-guts grown from patients. To understand the effects of aspirin, metformin, or a combination of both drugs, they exposed the cancer cells and mini-guts to these medications. They then used different methods, like qRT-PCR (a technique to measure gene activity), RNA sequencing (studying the genetic code), and clonogenicity assays (evaluating cell growth) to see how the drugs affected specific targets.

Once they identified potential targets, the researchers planned to validate their findings in translational studies, which involve applying these insights to real-world situations, potentially moving closer to practical applications or treatments for bowel cancer.

## WHAT WERE THE RESULTS AND WHAT DO THEY MEAN?

- **Key finding 1: Aspirin can reverse harmful changes in cells which occur during bowel cancer progression**

This work showed that aspirin reverses the cystic phenotype (Figure 1) and invasive properties such as epithelial to mesenchymal transition, that are driven by the Wnt signalling pathway by increasing a molecule (Dickkopf-1) that is a Wnt blocker and is often lost during bowel cancer progression (Figure 2- Summary graphical abstract from publication)

## WHAT WERE THE RESULTS AND WHAT DO THEY MEAN (continued)?

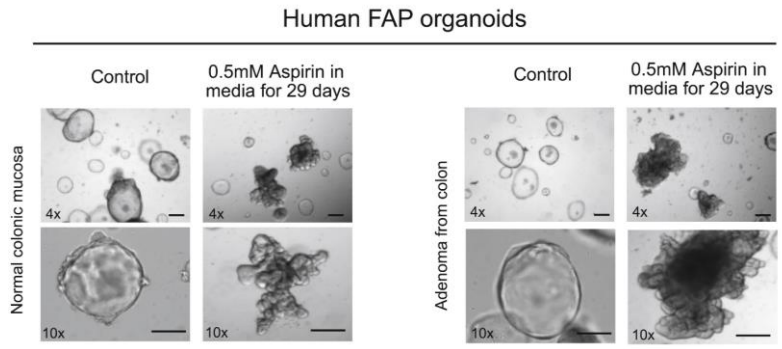



**Figure 1. Aspirin reduces the Wnt-driven budding phenotype in human organoids.** Brightfield images of organoids derived from human familial adenomatous polyposis (FAP) normal colonic mucosa or colonic polyp tissue exposed to low concentration aspirin 29 days. See full Figure in publication.

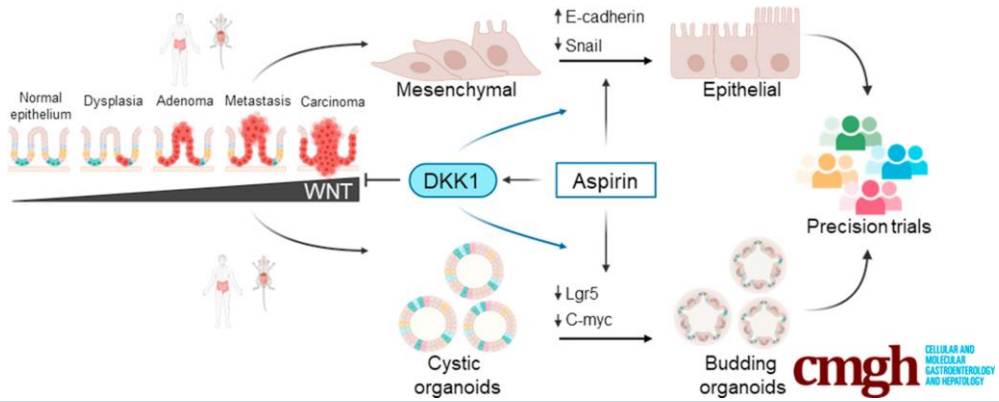



**Figure 2. Abstract from Cellular and Molecular Gastroenterology and Hepatology**, 2021,11 (2). Aspirin can reverse the Wnt-driven cystic phenotype in human organoids while reducing stem cell marker expression and increasing expression of Dickkopf-1, a Wnt antagonist often lost during CRC progression.

- **Key finding 2: Aspirin and metformin can reverse the increase in stem cells populations induced by o**ver-nutrition with certain nutrients Figure 3..

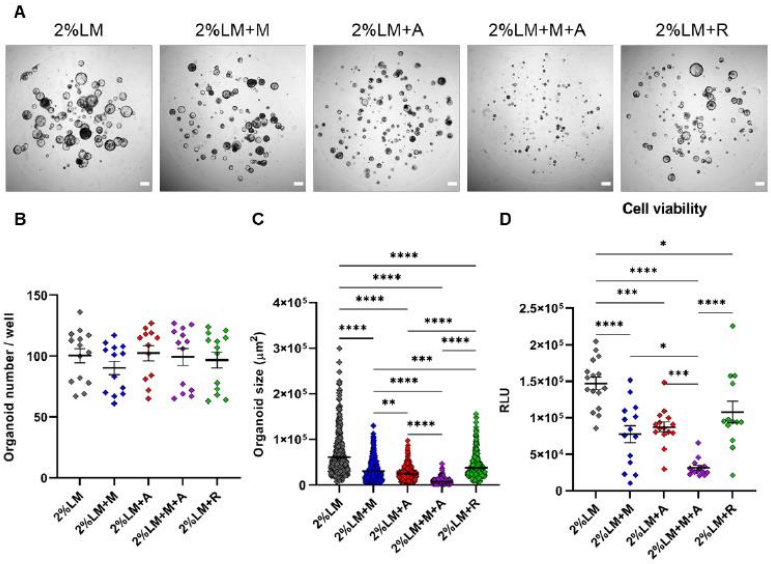



**Figure 3. Aspirin and metformin reverse the effects of overnutrition in Apcfl/fl organoids**. (A) Brightfield images of 2nd generation clonogenicity organoids treated with 2%Lipid mixture (2%LM) and 2mM metformin (2%LM+M), 2mM Aspirin (2%LM+A), 2mM metformin+2mM aspirin (2%LM+M+A) or 7.5nM Rapamycin (2%LM+R). Respective organoid (B) number and (C) size and (D) cell viability. Scale bars represent 300µm. Images were obtained using 2.5x magnification. Organoid size quantification was carried out using ImageJ software. Cell viability is shown as RLU of metabolically active cells. Error bars represent SD (n=3).P-values determined by One-way ANOVA, Tukey's post-hoc test (*=p<0.05; **=p≤0.01; ***=p≤0.001; ****=p≤0.0001).

- **Key Finding 3: Novel signalling targets identified in when bowel cells exposed to both aspirin and metformin compared to either drug alone.** The aim was to identify significant, molecular pathways whereby the combination of aspirin and metformin may have a beneficial role in bowel cancer prevention. We found several pathways that were modulated exclusively when cells were exposed both to aspirin and metformin ( Figure 4) and these were validated in cells and organoid models ( Figure 5)

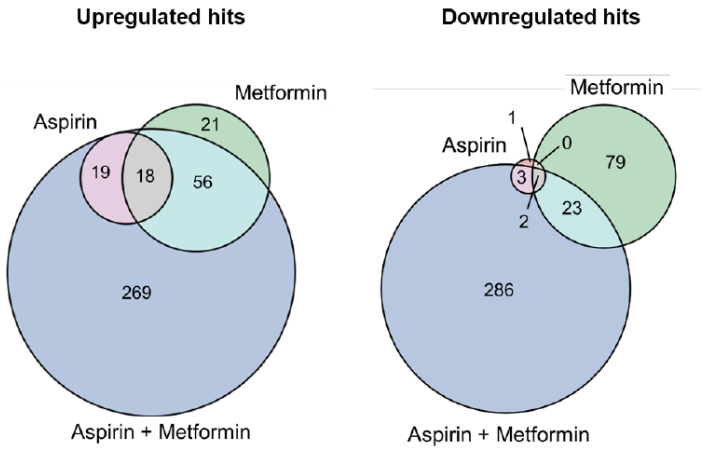



**Figure 4. Venn diagram of Lexogen RNA-seq targets in bowel cancer cells.**
HCT-116 cells were exposed to the drugs and then underwent RNA-sequencing to identify shared targets. Aspirin (red), metformin (green), aspirin+metformin (dark blue) as well as their overlaps aspirin-aspirin+metformin (pink), metformin and aspirin+metformin (light blue) and aspirin-metformin-aspirin+metformin (grey) .

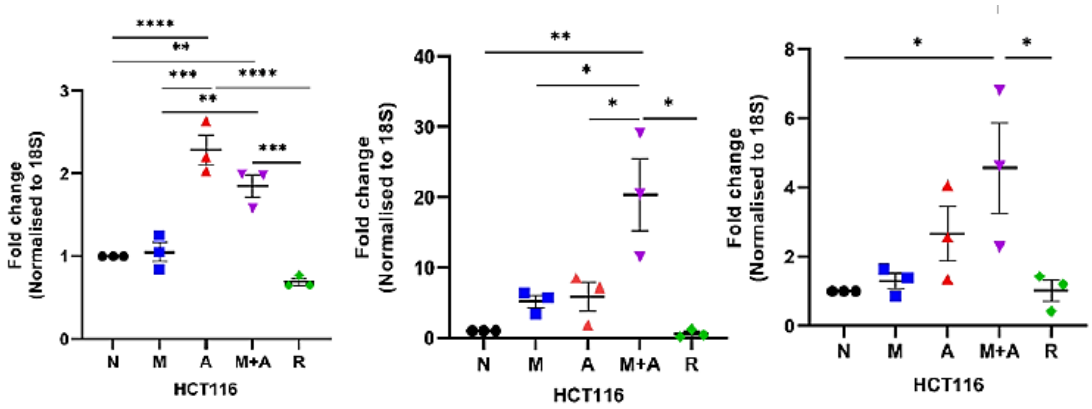



**Figure 5. Aspirin and metformin increase transcription levels of specific molecular markers in bowel cancer cell line ( HCT116).** HCT116 cells were treated for 24h with 3mM aspirin, 2mM metformin or their combination. Transcriptional levels of key genes identified from RNA-sequencing were measured by qRT-PCR. Data were normalized against 18S (n=3). Error bars represent SD. Statistical analysis was carried out on GraphPad using One-way ANOVA, Tukey's post-hoc test (*=p,0.05; **=p<0.01; ***=p<0.001; ****=p<0.0001).

## WHAT IMPACT COULD THE FINDINGS HAVE?

- **Patients-** Bowel cancer causes ~ 16,000 deaths in the UK annually and 694,000 deaths worldwide. Research which aids bowel cancer prevention is of major economic and societal impact. Whilst aspirin decreases CRC incidence and mortality, the understanding of the molecular mechanism is incomplete. These studies increased knowledge regrading how the drugs work and identifying potential biomarkers that determine whether the bowel lining of patients will respond favourably to potential cancer prevention agents.
- **Policy and practice-** Chemoprevention is a cost-effective approach for intermediate-risk populations following removal of bowel polyps. The key remains identifying which populations will respond. Our research, by identifying targets at a cellular level, may be able to inform larger scale polyp prevention studies and in time help policy makers in shaping chemoprevention strategies.

## HOW WILL THE OUTCOMES BE DISSEMINATED?

The results of this work have already been disseminated at several regional and national meetings ensuring cross-disciplinary academic beneficiaries can access results. The work has also been presented at collaborative networks (chemoprevention, organoid and CRUK Scotland) to maximise impact. Data have been published in peer-reviewed journals and further validation is being undertaken as part of new manuscript submissions.

## CONCLUSION

The CSO funding has allowed our research team to undertake important studies to define biomarker response profiles to aspirin and metformin that indicate whether a person may benefit from the cancer prevention properties of these drugs. This research is highly relevant to the long-term goal of bowel cancer prevention, a national health priority research theme.

## RESEARCH TEAM & CONTACT

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