FXR Regulates Intestinal Stem Cells Response to Bile Acids in a High Fat Diet

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Running title: FXR regulates intestinal stem cells in response to bile acids

Key words: FXR, stem cells, colon cancer, high fat diet, bile acid

Introduction

Although a high fat diet (HFD) is known to be associated with a poor clinical outcome for colorectal cancer (CRC) patients, the precise mechanisms underlying this are poorly understood. Fu and colleagues have recently identified high levels of bile acid in a preclinical mouse model of colon cancer, and that a high fat diet (HFD) increases bile acid (BA) further which in turn promotes proliferation in Lgr5+ stem cells, via inhibition of farnesoid X receptor (FXR), to drive tumour progression [1].

In addition to smoking and UV exposure, lifestyle factors such as diet, nutrition and physical activity, have been shown to play a significant role for many cancers, particularly colorectal cancer (CRC)[2]. CRC is the 3rd most frequent type of tumour and the 2nd leading cause of malignancy-related deaths in the Western world [3, 4]. Worldwide CRC incidence is increasing in female patients, those younger than 50 years-old and in low/middle income nations[3, 4]. It is estimated that ~50% of CRC cases annually are preventable through lifestyle and dietary changes[2, 5], with the presence or absence of certain dietary components strongly associated with an increased or decreased risk [2, 5]. Thus, to implement prevention measures for CRC we need to understand the mechanisms by which the environment impacts upon the normal biological processes of the intestine and how this affects
health and disease. The recent paper by Fu et al now provides insight into how a HFD impacts on intestinal stem cell activity to promote intestinal tumour growth [1].

**HFD induced changes to the ISC pool increases their vulnerability to oncogenic transformation.**

The epithelial surface of the intestine is maintained by a pool of rapidly dividing stem cells (ISCs), marked by expression of Lgr5, that reside in the intestinal crypts and have been demonstrated to be the cells of origin for CRC[6]. In 2016 Beyaz et al, [7] reported that a HFD elevated the number, proliferation rate and ability of the Lgr5+ intestinal stem cells and their progenitors to form 3D organoids in *ex vivo* culture. They demonstrated that a HFD diet induction of ISC activity was linked to upregulation of the canonical Wnt signalling pathway via the nuclear nutrient sensing receptor PPAR-δ. Canonical Wnt signalling plays a key role in gut development [8], homeostasis [9] and regeneration [10] and is the most commonly deregulated pathway in CRC; often due to inactivation of the APC gene[6, 11]. A HFD was shown to induce erosion of the intestinal crypt-villus structures exposing the ISCs and their progenitors to fatty acids within the diet. This instigated a fatty acid PPAR-δ dependent response within the crypt cells that enhanced their expression of canonical Wnt target genes thereby increasing their stemness and tumourigenicity[7]. This work provided a plausible cellular and molecular mechanisms for how an excess of dietary fats remodels the intestine and increased the number of cells which are capable of propagating a tumour following an oncogenic event [12]. Fu *et al* [1] have now addressed some of the questions that remained regarding how the response mechanisms to dietary fat alter intestinal biology, mediators of stem cell behaviour and impact on tumour initiation and progression.

**Increases in bile acids due to a HFD drive oncogenic transformation of ISCs**
Fu *et al.*, [1] demonstrated that increasing BA levels were commensurate with tumour load in the *Apc*<sup>min/+</sup> mouse, a model of the most common CRC related syndrome familial adenomatous polyposis (FAP). Addition of a HFD further increased BA levels and exacerbated the *Apc*<sup>min/+</sup> phenotype; likely linked to an impaired intestinal epithelial barrier. They then investigated the functional role of BAs in tumourigenesis by taking a reductionist approach and focussing on the primary tauro-β-muricholic acid (T-βMCA) and secondary BA deoxycholic acid (DCA). These BAs showed ~3-fold increases in response to a HFD in WT and *Apc*<sup>Min/+</sup> mice, increasing dramatically (~60 fold) in tumour bearing mice; demonstrating a synergy between HFD and tumours in mice. By administrating T-βMCA or DCA alone to *Apc*<sup>min/+</sup> mice they could replicate the BA serum levels, intestinal pathological changes and enhanced progression of intestinal tumourigenesis observed in HFD fed mice. Wnt target genes, and ISC markers, including *Lgr5*, were upregulated in BA treated mice suggesting ISC activity was increased by BA, and this was associated with inhibition of FXR by BA. Organoids cultured from *Apc*<sup>Men/+</sup> mice showed an increase in growth in response to BA which was associated with increased ISC marker expression, supporting the role of BA in activating ISCs. As T-βMCA has a known role in inhibiting the target genes of FXR, a primary sensor of nutritional cues that translates the stimuli into transcriptional programmes, they next examined if FXR regulated ISC activity. FXD agonists blocked the upregulation of ISC markers by BA in organoids cultured from *Apc*<sup>Min/+</sup> tumours, which was associated with reduced tumour organoid growth, whilst genetic deletion of FXR in tumour organoids increased growth, demonstrating a regulatory role for FXR between BA and ISC activity.

This work provides new, important insight into the links between HFD and CRC progression, and potentially reveals new therapeutic targets and will provide evidence to support public health advice on the risks of a diet high in fat.

**Outstanding questions over HFD induced ISC changes and CRC risk**
To support the importance of the BA:FXR axis in CRC, Fu et al examined if pharmacological activation of the FXR, using the specific agonist FexD, was able to inhibit intestinal tumour initiation/growth as a potential therapeutic strategy. However, interestingly the most dramatic response to this approach was in \textit{Apc}^{Min/+} mice on a normal diet (~40% reduction in tumour number), whereas the \textit{Apc}^{Min/+} mice on a HFD only displayed a modest ~25% reduction in tumour numbers. This is a surprising result given the dramatic reduction in BA in \textit{Apc}^{Min/+} mice on a HFD compared to only a partial reduction of BA in the \textit{Apc}^{Min/+} mice on a normal diet when treated with FexD. If BA is responsible for the increased tumour progression in \textit{Apc}^{Min/+} mice on a HFD, why do \textit{Apc}^{Min/+} mice on a HFD only show a 25% reduction in tumour numbers despite BA levels reduced almost to WT levels?

Furthermore, FexD treated mice show a convincing inhibition of tumour progression from adenoma to adenocarcinoma in \textit{Apc}^{Min/+} on a HFD, whereas the numbers of hyperplastic lesion and adenomas remain very similar between FexD and vehicle treated mice. One explanation could be due to intestinal permeability being twice as high in the FexD treated \textit{Apc}^{Min/+} mice on a HFD compared to those on a normal diet, and this is supported by the observation that the larger tumours are more sensitive to FexD in the HFD \textit{Apc}^{Min/+} mice, whilst in the \textit{Apc}^{Min/+} mice on normal diet the smaller tumours show the best response to FexD. Thus, the intestinal permeability may not have been improved sufficiently by FexD treatment in the HFD \textit{Apc}^{Min/+} mice to suppress tumourigenesis, whilst a normal diet permits lower levels of barrier dysfunction and consequently there are less smaller tumours developing. These data also suggest additional mechanisms mediate the response of ISCs cells to HFD which warrant future research into this important topic. An additional mechanism regulating tumour initiation/progression by the HGD/BA/FXR axis could be the effects of epigenome alterations[13, 14] or FexD inhibiting cytokines, including IL6, as the JAK/Stat pathway is rate limiting for Wnt and inflammation associated colon cancer [15, 16]).

Finally, while it is entirely plausible that increasing ISCs can lead to increased CRC risk, the picture is still not clear. The argument put forward for the link between a HFD and CRC risk is based on the premise that a HFD increases the number and activity of ISCs and cancer ISCs. Whilst inhibiting
cancer stem cell activity is highly plausible as a mechanism to reduce tumour growth/progression, the effect of an expansion of normal ISCs is a little more unclear in terms of increasing CRC risk. For example, although an increase in the number of normal ISCs will provide more cells that can undergo an oncogenic insult and initiate a tumour it doesn’t necessarily mean that it will increase risk. ISC numbers are maintained by a stochastic process called neutral drift in which each ISC has an equal probability of replacing its neighbouring ISC or being replaced. [17, 18]. However, an ISC with a CRC related mutation (e.g. partial or complete Apc loss) follows a biased drift model, as it has an increase in clonal fitness which favours its retention[19-21]. Thus, it follows that increasing the activity of normal ISCs, for example due to a HFD, could actually decrease the fitness advantage of a single pre-neoplastic mutated ISC thereby increasing the chances that it is lost. As the opposite has been shown to be true; inhibiting Wnt signalling decreased the number of WT ISCs thereby increasing the fitness of Apc mutant ISCs and increasing their fixation rate with a crypt [20, 21]

**Summary**

One of the main obstacles to public enthusiasm to modify their diets is a lack of faith in the advice given, and alterations in which diets are beneficial for health and why. Question such as “how does eating/not eating a certain dietary product impact my health?” are difficult to answer in a general context. This study by Fu et al helps build a robust body of knowledge to identify how diet impacts biology and health. While there are merits in taking a reductionist approach and using mouse models to investigate the effects of a single dietary component on ISCs translating the outputs to the real world will require a more holistic human focussed approach. As recent reports indicate that the absence of “healthy” dietary components can be as detrimental as the increased presence of “unhealthy” ones in terms of increasing cancer risk [4]. Each individual’s diet and microbiome are unique and deciphering how that impacts on ISC and CRC risk at a population level is a major challenge we have to meet if we are to reduce cancer incidence around the globe.
Acknowledgments

We kindly acknowledge the following funders: BLS/CMU Fellowship (TJP), MRC (TJP), AMMF (TJP), CRUK (LP) World Cancer Research Fund (LP), Wales Cancer Bank (LP) and Tenovus (LP). The authors declare no conflicts of interest.

References