

Reviewer 2

A well written interesting article. Recommend accept with minor revisions. This is an interesting animal model for UC - in that MUC2 mutant Winnie mice don't produce mucus but this is different from UC which has goblet cell depletion not MUC2 mutation. However, I believe this animal model is acceptable, but the authors require in the discussion why this animal model was used, and more detail as to MUC2 mutation in UC and in human disease.

1. *The aim - I believe it should be changed to: To determine if exacerbation of pre-existing chronic colitis in Winnie (MUC2 mutant) mice induces colonic dysplasia.*

Aim has been changed as suggested.

2. *Results need to focus on the fact that exacerbation of colitis in MUC2 mutation Winnie mice resulted in greater incidence of dysplasia when compared to mice without mutation*

Added sentence describing higher incidence to the abstract.

3. *Conclusion: I believe should be changed to: Alterations to the expression of Cav1, Ccl5, Myc, Trp53 in the chronically inflamed Winnie colon may influence the transition to dysplasia*

Changed conclusion as suggested.

4. *Core tips - should add 'This study demonstrated that exacerbation of colitis resulted in...*

Added suggested to core tip.

5. *Following the line '... it may not reflect the mucus abnormalities observed in UC' this should be followed by what the mucus abnormalities are in UC. Is there a better animal model than MUC2 mutant mice?*

Lines added as per the suggestion. Additionally, In our opinion, Winnie mice seem to recapitulate a number of features seen in UC patients. Hence, we believe Winnie, with just a frameshift mutation in MUC2 rather than the whole deletion better represents UC.

6. *There were no cancers (all animals killed day 42). Would there be benefit in a longer study to see if dysplasia progressed to neoplasia?*

In data not published, we have observed no benefit with a long term study (so far –up to 24 weeks) as the Winnies on DSS do not develop frank tumour lesions.

7. *In the methods section - make clearer the number of mice used in each group in the description*

Details of numbers of mice allocated to which group have been added to the methods.

8. *What is caecocolic junction? Was this meant to be ileocaecal junction?*

Yes, caecocolic has been replaced with ileocaecal.

9. *The results section needs to be made clearer - with the focus on the number of hyperplasia, dysplasia in each group.*

Have modified the results section to better report numbers of lesions with reference to Table 1.

10. *Discussion must have a review on the mucus changes in UC and whether this animal model can be used to reflect UC. I think it is appropriate, but would like to know what the literature says. Please make appropriate changes to manuscript and abstract.*

We have now re-written the opening of the discussion to better relate the choice of model to mucus changes in UC.

Reviewer 3

The current study by Randall-Demillo et al, aims to examine whether the loss of MUC2 and subsequent ER stress in the Winnie model is sufficient to drive colonic dysplasia with DSS challenge. The study gives insight into changes in the inflamed colon that may predispose to CRC, particularly relevant to patients with ulcerative colitis and will likely be informative for future studies investigating the links between IBD inflammation, ER stress and CRC. The manuscript would benefit from careful proofreading, as there are a number of typos (eg extra "colonic" in the Aims).

There are a number of questions and comments that should be addressed, which would improve the manuscript for publication:

Questions/comments the hypothesis, as written, is vague and unclear "a similar long-standing, low-level colitis in Winnie mice could accelerate development of colonic adenocarcinoma" -similar to what?

Yes, this was vague. Have changed the wording to "defective mucus layer and resulting chronic colitis in Winnie would increase the incidence of colonic adenocarcinoma" to better clarify the hypothesis.

Were WT animals litter matched? Both genders of mice were used, given CRC is more prominent in males, was any gender difference observed?

While not mentioned in the initial draft of the manuscript, wild-type littermates were randomly allocated to either treatment group. While both male and female animals were included, it was not possible to detect any difference between sexes.

Affymetrix DSS is the correct Molecular Wt but is reported as more variable than MP Biomedical DSS. Have the authors compared sources, or increased Affymetrix DSS concentration?

Although we have not compared the response induced by DSS from USB-Affymetrix with that of other manufacturers under our conditions, results from Bamba et al. (2012) indicate that the effects induced by DSS sourced from USB-Affymetrix and MP Biomedicals are indistinguishable. Our decision to use 1% DSS was based on previous results using two cycles of 2.5% DSS in Winnie after Heazlewood et al. (2008). While we acknowledge that the optimal concentration of DSS used would ideally be determined empirically, we selected a two-fold reduced concentration of DSS so as to reduce mortality for the longer DSS protocol used in the present study.

Why not do a longer study? Why not use weight/length ratio? Why have colon mass/length at all?

Page 11 - Histopathology - Please clarify the sentence "Crypt hyperplasia DSS administration also induced a heavy influx of leukocytes into the mucosa and submucosa" What is "Crypt hyperplasia DSS administration"?

Misplaced "crypt hyperplasia" deleted. "Heavy influx" changed to "increased influx"

Page 11- histopathology - The text "In 27% of Winnie mice exposed to DSS, crypt epithelium could be observed within the submucosa underlying apparently dysplastic lesions separated by an intact muscularis mucosae" should refer to table 1.

Missing reference to table 1 added.

For Table 1, was statistical analysis performed? This should be included and defined.

Statistical analysis was performed on Table 1. Have updated the results to include analysis.

A short rationale for each results section would help the reader understand the experimental process.

Yes. Agreed. We have now included short rationale for results

Fig 1 A is difficult to interpret. The authors should consider either 1) removing colors and enlarging data point symbols with alternative open/solid symbols for WT vs Winnie OR 2) displaying WT and Winnie side by side.

Figure 1A has been split into two figures (WT vs Winnie) to improve the readability.

Given that mucosal mass is a better indicator of inflammation than colon shortening and data is available, the authors may consider using weight/length ratios rather than length alone.

Fig 1C and 1D are not discussed in the text and it is not clear what benefit the information associated with Fig 1C provides.

Included the missing reference to Figure 1D. Since Figure 1C was not providing much information, it has been omitted.

Fig 2, the definitions of PC, MC and DC should be provided in the figure legend. Fig 2 is again, difficult to interpret as presented, outlining the dot points with a column bar maybe be helpful.

Figure 2 plot has been laid over summary columns for each colonic segment. Definitions of PC, MC and DC have been added to Figure 2 legend.

Fig 6 and Fig 8, are equivalent WT images available, these should be included?

The rationale of targets for gene expression analysis is a little unclear. The authors state they are "potentially involved" but no citation is given or rationale for the selection vs the myriad of other genes. A list of citations or table outlining the involvement would be helpful. "all other genes tested" Which genes? How many? With that in mind, a comparison of Winnie-DSS with AOM-DSS may be useful, with respect to dysplasia-associated genes. A comprehensive list of gene changes were outlined by Gao et al in a 2013 Carcinogenesis article (doi:10.1093/carcin/bgt135)

It is interesting that the MUC1 response to DSS was lost in the Winnie mouse, have the authors any hypothesis on the significance of this? fig 7, it is a little unclear what the significance marker for Cav-1 represents. The results section suggests an increase between control and DSS WT mice, but the graph suggests Winnie DSS? Have the authors examined the distal ileum? In the C.

rodentium model loss of small intestinal glycosylation can lead to invasion of the normally resistant ileal mucosa by *C. rodentium*. Loss of MUC2 may similarly leave the ileum vulnerable to DSS insult and dysplasia. In addition, Martin et al (Plos One, 2011 - <http://dx.doi.org/10.1371/journal.pon>

Given that an ineffective inner mucus layer could permit increased bacterial interaction with the Muc1 external domain due to and thus alter NF- κ B-dependent and p53-dependent signalling in the epithelium, Muc1 would make an interesting target for study in Winnie. However, we were unable to detect a change in Muc1 mRNA in the present study.

The distal portion of the colon is the site of most neoplasms in the lower gastro-intestinal tract in humans. Although cancers arising in the small intestine are commonly reported in common strains of laboratory mice with Apc^{Min/+} mutation, human small intestinal cancers are exceedingly rare. To our knowledge, no convincing association has been made between chronic ileitis and increased incidence of ileal neoplasia. For this reason the present study concentrated on the distal colon alone and the terminal ileum was not examined.