

Reply to the reviewers

Reviewer 03262698

Reviewer's comment:

The work is interesting however I have some suggestions:

1. Introduction (line 6): please clarify and add reference to “prevent the development of drug resistance”.

Reply: We deleted this sentence because this was our hypothesis but not yet clearly proved.

Reviewer's comment:

2. Results: in the section “Fn14 KO mice are resistant to the side effects of 5-FU” I suggest to remove the first sentence “Diarrhea is a critical indicator of chemotherapeutic agent-induced adverse effects in the intestine.”

Reply: We deleted this sentence.

Reviewer's comment:

3. Results: Please check: figure 1 A, B and so on cited in the text do not correspond to the figure at the end of the file.

Reply: Thank you for this comment and we corrected the text.

Reviewer's comment:

4. Results: There are some data not shown that should be added (also in the method section, i.e. apoptosis, proliferation and expression of transporter genes related to diarrhea).

Reply: These data were added as Supplementary Materials of the revised manuscript. Method was also provided in the Materials and Methods.

Reviewer's comment:

5. Results: I suggest to add a subparagraph in the results on IL-13R α 1 KO mice, making a separate figure for data of Figure 3B and C

Reply: We separated the data from Figure 3 to a new Figure 4, and accordingly revised the numbers of following figures.

Reviewer's comment:

6. Discussion: I suggest to move from “Fn14 is often upregulated in malignant tumors” (pag 19)... to ... “Careful investigation of the effects of blocking TWEAK/Fn14 signaling on each cancer cell type is needed.” (pag. 20) at the beginning of the discussion.

Reply: We moved these sentences to the second paragraph of Discussion.

Reviewer's comment:

7. Discussion: I suggest to change the first sentence: “Blocking the TWEAK/Fn14 pathway suppressed 5-FU-induced diarrhea.” In “Blocking the TWEAK/Fn14 pathway suppressed 5-FU-induced diarrhea, but did not affect tumor growth in the CT26 tumor-bearing model or the antitumor effects of 5-FU.” (from the sentence at pag. 19 “In our study, blocking the TWEAK/Fn14 pathway did not affect tumor growth in the CT26 tumor-bearing model or the antitumor effects of 5-FU.”).

Reply: We changed the sentence as indicated.

Reviewer's comment:

8. Discussion: I suggest to remove the sentence “Additionally, Fn14-deficiency could result in lower levels of other inflammatory cytokines, such as TNF- α , IFN- γ , and IL-17A, which also might affect IL-33 cleavage by inflammatory cells. Further studies are needed to investigate IL-33 processing, cellular localization, and function.”

Reply: We deleted the sentence as indicated.

9. Figure legends/statistics: different tests have been applied (Two-way ANOVA with Bonferroni correction, one-way ANOVA, Students t test, Mann Whitney test), this choice must be justified and significance of each time versus day 0 within single group and of KOmice versus WT within single time must be showed.

Reply: According to this comment and the result of the review by a biological statistician, we revised some description of statistical analysis. In summary, all statistical methodologies are now described in statistical analysis section. $P < 0.05$ was considered to be statistically significant and indicators in graphs were simplified. Results obtained from three or fewer samples were not subjected to statistical analysis, but our observation was mentioned. Reason of the loss in observation was explained in the figure legend. The word “significantly” is now used with P value, or deleted where it is not necessary. These revisions are listed below. Overall these revisions did not change the scientific results and interpretation in our original manuscript.

Fig 1B, Diarrhea score. In the graph, all indicators of statistically significant difference were changed to asterisks. The reason for loss of observation was mentioned in the figure legend as followed; “Number of mice was ~7-44 at each time point. Results of mouse groups of different observation-time points and endpoints were accumulated and summarized.” Statistical method was described in section of “Statistical analysis.”

Fig 1E and 1F, mRNA levels. Statement of statistics was deleted from the legend because of number of the samples were 3.

Fig. 2C, Diarrhea score. Raw P value was inserted in the text. Statistical method was described in section of “Statistical analysis.”

Fig. 3, Cytokine measurement. In the graph, all indicators of statistically significant difference were changed to asterisks. Statistical method was described in section of “Statistical analysis.” “Significantly” was deleted from the text, because it was not necessary.

Fig 4B, Diarrhea score. In the graph, all indicators of statistically significant difference were changed to asterisks. The reason of loss of observation was mentioned in the figure legend; “Number of mice was ~5-44 at each time point. Results of mouse groups of different observation-time points and endpoints were accumulated and summarized.” Statistical method was described in section of “Statistical analysis.”

Fig5A, Cytokine measurement. In the graph, all indicators of statistically significant difference were changed to asterisks. Method was described in section of “Statistical analysis.” In addition , labeling of X axis was not correct in original figure and now corrected.

Fig 5D, Cell numbers. Statement of the results from ANOVA were deleted from the graph and

legend, because the number of the samples was 3.

Fig 6A, mRNA levels. Statement of statistics was deleted from the graph and legend, because the number of the samples was 3.

Fig. 6C, protein expression levels. In the graph, an indicator for statistically significant difference were changed to asterisks. Statistical method was moved from the legend to the section of “Statistical analysis.”

Fig. 6E, protein expression levels. Statistical method was moved from the legend to the section of “Statistical analysis.” Since the results were not statistically significant, there is no revisions in the text.

Fig. 7A, protein expression levels. In the graph, all indicators of statistically significant difference were changed to asterisks. Statistical method was described in section of “Statistical analysis.”

Fig. 7C, protein expression levels. In the graph, all indicators of statistically significant difference were changed to asterisks. Statistical method was described in section of “Statistical analysis.”

Reviewer 03262112

Reviewer’s comment:

This article concerns with a complete, innovative, very well defined and structured molecular study with important consequences on common clinical oncological practise.

A molecular strategy is elaborated to prevent common 5FU adverse effect like anorexia and diarrhoea, symptoms that can reduce patient's compliance to chemotherapy.

The manuscript presentation is clear, readability is very good, very good English level.

Author contribution, Grant support, animal care are well defined in the title page.

The **title** reflects the major topic and content of the study; it is focused and synthetic.

The **Abstract** is well structured and clearly defines the study, respecting the sections and with a clear synthesis.

The **manuscript** defines every detail of the study: the reader is provided of all information to completely understand the matter even if surgeon (like me!).

Every section is really appropriated into its matters: intrioudction, materails and methods well describe in detail the experimetal study.

Pathology is well descripted with attention to the topic.

Discussions give complete argumentation on the basis of literature in terms of recent researches: the analysis of literature is complete and exhaustive.

Figures are original, reflecting an original and well structured study; they are well explained into legends.

The **references** are appropriate, relevant and up to date.

In my opinion the manuscript should be accepted.

Great best regards and thank You all for the confidence.

Reply: We appreciate this comment.