Peer Review File

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Reviewer A

This manuscript assesses the prognostic value of ITLN1 in colorectal carcinoma. There is interesting information here, but I have several comments.

Comment 1: * Why is COAD (colonic adenocarcinoma) used sometimes, and CRC (colorectal carcinoma) used other times?

Reply 1: We sincerely thank you for your valuable comments. According to the relevant literature, the main type of CRC is Colon adenocarcinoma (COAD), develops from gene mutations in adenomatous lesions. COAD project in The Cancer Genome Atlas (TCGA) database served as the training cohort, we used this dataset to obtain the results of this study to assess the value of *ITLN1* in CRC. The same is true in related studies (1,2).

Changes in the text: As suggested, CRC is used to summarize and discuss the existing results in this manuscript, and COAD is used to express the results obtained using TCGA database. We have synchronously revised the manuscript to fully address this issue (see Page 2, line 25; Page 6, line 95).

References

1. Yan G, An Y, Xu B, et al. Potential Impact of ALKBH5 and YTHDF1 on Tumor Immunity in Colon Adenocarcinoma. Front Oncol 2021;11:670490.
2. Huang C, Ou R, Chen X, et al. Tumor cell-derived SPON2 promotes M2-polarized tumor-associated macrophage infiltration and cancer progression by activating PYK2 in CRC. J Exp Clin Cancer Res 2021 ;40:304.

Comment 2: * The authors mention that "several studies" have shown ITLN1's use in CRC, but they never elaborate on the findings in these studies. This should be part of

the Discussion section.

Reply 2: Thank you for your constructive comments on my manuscript. "Several studies have shown that intelectin-1 (*ITLN1*) can serve as a key prognostic and therapeutic target for colorectal cancer." in the background of the abstract, has been elaborated in sections in the Discussion. The list is as *Table 1* in the Response to Reviewers.

Table 1. *ITLN1* is a key prognostic and therapeutic target for colorectal cancer.

ID.	several studies (Page xx, line xx)	References	research results
1	Page 15, line 286-295	(28-30)	a low expression level of *ITLN1* leads to dysregulation of the PI3K/Akt pathway (28); The PI3K/Akt pathway is an intracellular signalling pathway related to proliferation, differentiation and apoptosis and is an important pathway for body self-protection (29); *ITLN1* can reduce the level of secondary bile acid by inhibiting bile secretion in CRC patients, thus achieving cancer inhibition (30).
2	Page 16, line 300-304	(16,17)	*ITLN1* can reduce the malignant behaviour of CRC cells, as indicated by cell growth, metastasis and invasion, and that decreased *ITLN1* expression is independently associated with the progression and poor prognosis of CRC (16); Kim et al. identified intelectin-1 as a marker of favourable outcome in stage IV colorectal cancer patients (17).

Comment 3: * ITLN1 may be more expressed in normal colon than in CRC, but that does not make it a "diagnostic biomarker." Distinguishing normal colon from CRC is easy microscopically. Distinguishing high-grade dysplasia from CRC on biopsy can sometimes be challenging, however. This is not investigated in this study.

Reply 3: We have carefully considered the suggestion of reviewers and make some changes. "diagnostic biomarker" has now been changed to "valuable tool" (see Page 2, line 37; Page 14, line 272), and "diagnostic assistance reference tool" (see Page 11,

line 196).

We agree that distinguishing high-grade dysplasia from CRC on biopsy can sometimes be challenging, but at present, the corresponding data of high-grade dysplasia has not been shared in the international database for research. In fact, this is an exciting area for future research. It is also our future research goal.

Changes in the text: "diagnostic biomarker" has now been changed to "valuable tool" (see Page 2, line 37; Page 14, line 272), and "diagnostic assistance reference tool" (see Page 11, line 196).

Comment 4: * Line 107: "male or female" is a useless inclusion criterion, as that covers nearly everybody.

Reply 4: Thank you for your reminder. We apologize for our careless mistakes. “were male or female”has now been changed to“male and female half” (see Page 7, line 112-113).

Changes in the text: “were male or female”has now been changed to“male and female half” (see Page 7, line 112-113).

Comment 5: * Why were only 10 cases stained for ITLN1? That is a small number and not very helpful.

Reply 5: We think this is a good suggestion, only 10 cases stained for *ITLN1* in the original manuscript were not very convincing. The preliminary literature survey made us decide on the preliminary experimental plan of using 10 cases stained for *ITLN1* (1). Therefore, a total of 10 cases stained for *ITLN1* were made and presented in the manuscript from getting the approval number of the ethics report to submission. After submission, we conducted another 20 staining experiments, and the IHC scores of a total of 30 patients were shown in *Table 2* in the Response to Reviewers, which was consistent with your professional suggestions.

References

1. Wu D, Xiang L, Peng L, et al. Comprehensive analysis of the immune implication of FABP4 in colon adenocarcinoma. PLoS One 2022;17:e0276430.

Comment 6: * How was IHC scoring assessed? There is no metric given. I therefore cannot truly assess how staining compared in normal colon versus CRC.

Reply 6: We sincerely appreciate the reviewer's professional suggestion. According to the suggestions of the reviewers, we provided the corresponding IHC scores of 10 patients in the early stage as the *Table S1* of the manuscript (see Page 11, line 199), and the IHC scores of a total of 30 patients were shown in *Table 2* in the Response to Reviewers. IHC scores calculation method see Page 8, line 126-132.

Table 2. Immunohistochemical staining score.

No.	tumour			normal		
	intensity of immuno staining	the percentage of positive tumour cells	weighted score	intensity of immuno staining	the percentage of positive tumour cells	weighted score
1	1	2	2（-）	3	4	12（+++）
2	1	1	1（-）	3	3	9（++）
3	1	2	2（-）	3	4	12（+++）
4	0	1	0（-）	3	4	12（+++）
5	1	3	3（+）	3	4	12（+++）
6	0	1	0（-）	3	3	9（++）
7	1	2	2（-）	2	4	8（++）
8	0	1	0（-）	2	3	6（++）
9	1	1	1（-）	3	4	12（+++）
10	1	3	3（+）	3	4	12（+++）
11	1	3	3（+）	3	3	9（++）
12	1	2	2（-）	3	4	12（+++）
13	0	1	0（-）	3	4	12（+++）
14	0	1	0（-）	3	4	12（+++）
15	1	1	1（-）	2	4	8（++）
16	1	1	1（-）	2	3	6（++）
17	1	2	2（-）	3	3	9（++）
18	0	1	0（-）	3	4	12（+++）
19	1	2	2（-）	3	4	12（+++）
20	1	3	3（+）	3	4	12（+++）
21	1	1	1（-）	3	3	9（++）
22	1	2	2（-）	3	3	9（++）
23	1	2	2（-）	3	4	12（+++）

24	1	2	2（-）	3	4	12（+++）
25	0	1	0（-）	2	4	8（++）
26	1	1	1（-）	2	4	8（++）
27	1	1	1（-）	3	4	12（+++）
28	1	2	2（-）	3	3	9（++）
29	1	2	2（-）	3	3	9（++）
30	1	2	2（-）	3	4	12（+++）

Changes in the text: According to the suggestions of the reviewers, we provided the corresponding IHC scores of 10 patients in the early stage as the *Table S1* of the manuscript (see Page 11, line 199). IHC scores calculation method see Page 8, line 126-132.

Comment 7: * Line 134: If 344 genes were assessed, should a Bonferroni correction be applied? Otherwise, P<0.05 should indicate that roughly 17 genes are 'statistically significant' just by random chance.

Reply 7: In response to professional suggestions from the reviewers, we revisited our manuscript. Although the P values of 344 genes described by Line 134 in the original manuscript have been corrected by Bonferroni. As shown in Figure 1 in the Response to Reviewers, the corrected pvalues have been arranged in descending order, far less than 0.05 (P<3.58×10-16). However, the screening method of *ITLN1* related genes is not clearly explained in the manuscript. In order to make the expression of the manuscript more detailed and scientific, we have made specific modification in the manuscript (see Page 11, line 188-189). Thank you again for your careful reading and guidance.

	A	B	C	D
1		cor	pvalue	bonferroni
2	ITLN1	NA	NA	NA
3	RTKN	-0.400554865	1.84E-20	3.58E-16
4	ZNF251	-0.400780181	1.74E-20	3.40E-16
5	FRZB	0.401071611	1.63E-20	3.17E-16
6	KRT20	0.401131262	1.60E-20	3.12E-16
7	DKC1	-0.401155786	1.60E-20	3.11E-16
8	MFSD6L	0.401624905	1.43E-20	2.78E-16
9	SNTB1	-0.402105108	1.27E-20	2.48E-16
10	SIDT1	0.402158963	1.26E-20	2.45E-16
11	VWA5A	0.402453902	1.17E-20	2.28E-16
12	CYSLTR1	0.402520683	1.15E-20	2.24E-16
13	ABCA8	0.403593547	8.92E-21	1.74E-16
14	RRP12	-0.403673811	8.75E-21	1.70E-16
15	TBC1D10A	0.40413344	7.83E-21	1.53E-16
16	BTD	0.404223044	7.67E-21	1.49E-16
17	CLC	0.40502	6.33E-21	1.23E-16
18	SLC51B	0.405071324	6.25E-21	1.22E-16
19	USP2	0.405490807	5.65E-21	1.10E-16
20	GREM2	0.405551692	5.57E-21	1.08E-16
21	NAT2	0.405877461	5.15E-21	1.00E-16
22	SPECC1	0.40605543	4.93E-21	9.60E-17
23	C10RF210	0.406152492	4.82E-21	9.38E-17
24	IRF4	0.406162406	4.80E-21	9.35E-17
25	CCL13	0.406226455	4.73E-21	9.21E-17
26	SGSM3	0.406853894	4.06E-21	7.91E-17
27	CCDC152	0.406965244	3.96E-21	7.70E-17
28	PALD1	-0.407390303	3.57E-21	6.95E-17

Sheet1 Sheet2 Sheet3

求和=0 平均值=0 计数=344

Figure 1. The P value after Bonferroni correction

Changes in the text: We have made specific modification in the manuscript (see Page 11, line 188-189).

Comment 8: * Line 172: The "several other R packages" should be listed/specified.

Reply 8: Thank you for your careful reading. We apologize for the inconvenience caused by the lack of detail in the manuscript. We have made additional explanations in the manuscript (see Page 10, line 184).

Changes in the text: We have made additional explanations in the manuscript (see Page 10, line 184).

Comment 9: * Line 194: ITLN1 expression may be "significantly lower" in the patients listed, but P-values are not given anywhere in the text or in Table 1 to confirm this.

Reply 9: Thank you for your reminder. We apologize for our careless mistakes. “significantly lower” in line 194 of the original manuscript has now been changed to“*ITLN1* expression tended to decrease in patients with advanced-stage disease, advanced-T classification, or advanced metastasis, although this trend was not statistically significant” (see Page 11-12, line 207-209).

Changes in the text: “significantly lower” in line 194 of the original manuscript has now been changed to“*ITLN1* expression tended to decrease in patients with advanced-stage disease, advanced-T classification, or advanced metastasis, although this trend was not statistically significant” (see Page 11-12, line 207-209).

Comment 10: * The first paragraph of the Discussion covers material more suited for the Introduction.

Reply 10: Thank you for your professional suggestion. We have reorganized the first paragraph of the Discussion according to the reviewer's suggestion. The first paragraph of the Discussion has been condensed and part of it has been put into the first paragraph of the Introduction (see Page 14, line 266-270; Page 4-5, line 57-60).

Changes in the text: The first paragraph of the Discussion has been condensed and part of it has been put into the first paragraph of the Introduction (see Page 14, line 266-270; Page 4-5, line 57-60).

Reviewer B

1. Are they the same hospital? If yes, please unify the name.

[2]The Second Affiliated Hospital of Wannan Medical College, Department of Pathology, Wuhu, 241000,

performed on samples from ten patients diagnosed with colorectal cancer who had undergone primary

surgery at the Affiliated Hospital of Wannan Medical College from August to November 2023, and

Reply 1: Thank you for your reminder. We apologize for our careless mistakes. "the Affiliated Hospital of Wannan Medical College" has now been changed to "the Second Affiliated Hospital of Wannan Medical College" (see Page 7, line 117).
Changes in the text: "the Affiliated Hospital of Wannan Medical College" has now been changed to "the Second Affiliated Hospital of Wannan Medical College" (see Page 7, line 117).

2. Please check if any more references need to be added in the below sentence since you mentioned "studies", but only one reference was cited. If not, "studies" should be changed to "a study/a previous study".

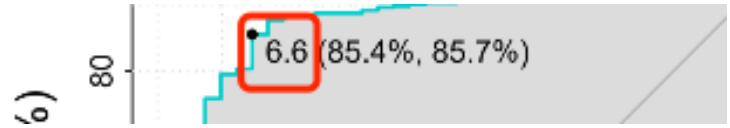
clinicopathological factors to predict survival in COAD patients. We found that tumour stage was the

most sensitive predictor, and previous studies have confirmed this (31). Therefore, in this study, all stages

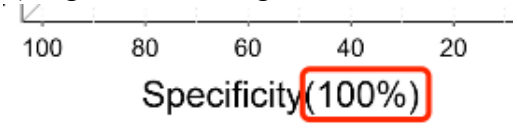
Reply 2: Thank you for your reminder. We apologize for our careless mistakes. "previous studies have confirmed this (31)" has now been changed to "a previous study has confirmed this (31)" (see Page 16, line 311).
Changes in the text: "previous studies have confirmed this (31)" has now been changed to "a previous study has confirmed this (31)" (see Page 16, line 311).

3. Table 1:
Please indicate the full name of "NA" in table 1 foot.
Reply 3: We sincerely appreciate the valuable comments. We have resubmited new version of Table 1. We added "NA, not available (represent missing value)" to the footnote of Table 1.
Changes in the Table 1: We added "NA, not available (represent missing value)" to the footnote of Table 1.

4. Figures:
1) Figure 1B and Figure 6B: Please indicate what the data means. Or add unit for the data.



2) Figure 1B and Figure 6B: Please revise "(100%)" to ", %" in the X/Y-axis.



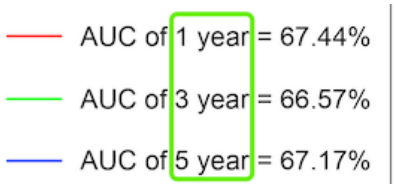
3) Figure 2A: Please add unit for Age.

4) Figure 4A: Please revise below words to “P value” and “Hazard ratio (95% CI)”.

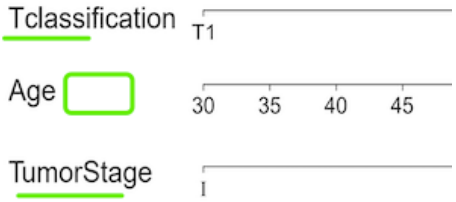
Pvalue	Hazard Ratios
0.02	0.921(0.859−0.987)
0.03	1.691(1.063−2.690)

5) Figure 4A: to standardize the results, the part that exceeds the horizontal coordinates should be indicated by arrow as below.

6) Figure 4F: Please revise below to “1-year, 3-year” 5-year”.



7) Figure 5A: Please add a space between the words and a unit for Age.



Reply 4: We have modified the figures according to editors' suggestion, and will submit the modified figures to you as attachments.

1) Figure 1B and Figure 6B: ROC analysis using R showed that the predictive value of maximum sensitivity and specificity was 6.6 (it was the optimal threshold for *ITLN1* expression in COAD, unitless), and could best distinguish normal tissue from COAD tissue.

Changes in the text:

1) Figure 1B and Figure 6B: “Moreover, the receiver operating characteristic (ROC) curve was used to evaluate the efficacy of *ITLN1* as a biomarker of COAD,” has now been changed to “Moreover, R package pROC was used to analyze the optimal threshold of *ITLN1* expression and the receiver operating characteristic (ROC) curve. The ROC curve evaluated the efficacy of *ITLN1* as a biomarker of COAD,” (see Page 8, line 137-139).

we added “(The optimal threshold of *ITLN1* expression was 6.6)” (see Page 11, line 198).

we added “the AUC of the *ITLN1* expression level was 0.91 (The optimal threshold of

ITLN1 expression was 10.2).” (see Page 14, line 260-261).