

Reply to reviewer # 1:

Thanks to the reviewers for his insightful advice and apologies for our carelessness. In response, we revised the manuscript and retouched the language of the entire manuscript in red. The replies to the seven questions are as follows:

1. We counted 92 patients with or without recurrence and the site of recurrence, and found 25 patients with significant disease progression, mostly with lung metastases (12 cases), liver metastases (9 cases), and 5 patients with concomitant or only bone metastases, in 1 case of ovarian metastasis, we analyzed the lung and liver of the recurrent sites and found no significant correlation between the recurrent sites and the expression of DCLK1/Lgr5 ($p > 0.05$), as shown in Table 1 and Table 2. Single factor regression analysis (table 3) was included, and recurrence and survival could not be predicted ($p > 0.05$).
2. All patients received postoperative adjuvant chemotherapy, 7 patients with T₃N₀ were treated with Xeloda alone, and the rest were treated with FOLFOX/XELOX regimen, which had no significant correlation with the expression of DCLK1/Lgr5 ($p > 0.05$), such as table 1 and Table 2.

3. We studied the relationship between preoperative Tumor Markers (CEA and CA19-9) and DCLK1/Lgr5 in 92 patients and found no significant correlation ($p > 0.05$), as shown in table 1 and Table 2.
4. Of the 25 patients who had relapsed, 2 patients refused treatment, the rest were kept on chemotherapy, 3 patients (rectal cancer) were treated with radiotherapy at the same time, 5 patients with oligometastasis (lung or liver metastasis) underwent resection of the lesion, 6 patients also received targeted therapy (Cetuximab or Bevacizumab).
5. Among the recurrent patients, 3 patients underwent pulmonary resection, 2 patients received hepatectomy, and 4 patients underwent radiofrequency ablation.
6. Until 2016, doctors at our hospital routinely don't perform K-RAS/BRAF test for patients with II/III, so those information could not be collected, MSI testing was expensive, and only a few patients were willing to do it, MSS Or MSI were estimated based on the results of dMMR immunohistochemical staining. We found no significant correlation between MSI and DCLK1/Lgr5 ($p > 0.05$), as shown in tables 1 and 2.

7. Preoperative Tumor Markers (CEA and CA19-9) were included in the univariate and multivariate analysis, the findings were suggestive of recurrence and survival (table 3) . The postoperative adjuvant chemotherapy was excluded from table 3 because the postoperative medication regimen was unevenly distributed in the whole case. Lung Metastasis, Liver Metastasis and MSI were included in Cox regression analysis (table 3) , suggesting that recurrence and survival could not be predicted ($p > 0.05$) .

Reply to reviewer # 2:

First of all, thank you for your helpful advice. As suggested, we revised the manuscript and colored the language of the entire manuscript in red. The answers to these two questions are as follows:

1. It is true that table 3 regression analysis and table 1 do not show all the relevant factors and may indeed be relevant to DCLK1/Lgr5. Therefore, the relationship between preoperative CEA and CA19-9, postoperative adjuvant therapy, recurrence, metastasis site, MSI and DCLK1/Lgr5 was analyzed, it was found that there was no significant correlation with the expression of DCLK1/Lgr5 ($p > 0.05$) . Table 1 and Table 2

showed that only the left colon and rectum, which were the primary sites of the tumor, accounted for a higher proportion of Lgr5^{Low} (76% VS 24% , 70% VS 30% , $p = 0.032$) .

2. In addition, we also included the above factors in table 3 for Cox univariate and multivariate regression analysis, and found that CEA and CA19-9 could indicate recurrence (HR = 4.853, 95% CI: 1.651-14.154, $p = 0.004$, and HR = 4.102, 95% CI: 1.529-11.005, $p = 0.005$) , CEA and recurrence could predict survival and had statistical significance (HR = 4.363, 95% CI: 1.346-14.136, $p = 0.014$, and HR = 12.002, 95% CI: 3.066-46.988, $p = 0.001$)