Reviewer A

These studies are vital because there is still controversy about prognostic factors in stage 2 colon cancer. Congratulations to the authors for the excellent research, and here's my opinion. This research focuses on the potential role of zinc finger protein, investigating its prognostic and predictive value, particularly in adjuvant chemotherapy.

The study employs a detailed screening strategy, combining quantitative proteomics and a nested case-control design. This approach lends credibility and thoroughness to the research. The results indicate a significant correlation between lower ZNF326 expression and reduced 5-year overall survival rates among stage II CRC patients.

However, it would have been nice to see a combination of known prognostic factors, such as high grade (differentiation) and obstruction, and to see which factor was more influential.

Do you know when the patients in the cohort were treated? The timing of treatment could be important, as there are differences in chemotherapy regimens, surgical techniques, etc.

Also, does distal vs. proximal in the classification mean colon, rectum? It needs more clarification.

Answer: Thank you for the suggestions and recognition from the reviewers. The main objective of this study was to identify predictive biomarkers for adjuvant chemotherapy in stage II colon cancer patients. However, due to limitations in the public database used for validation, some relevant variables that have been reported in previous literature to impact prognosis were missing. Nonetheless, our study employed standard research methods and rigorous statistical analysis, resulting in reliable and well-validated results in external databases. Our research team is currently conducting further studies with the expectation of achieving better research outcomes.

It is true that factors such as differentiation grade, obstruction, and surgical techniques were not included in this study, which could theoretically introduce bias to the results. However, ZNF326 expression was identified as an independent prognostic factor through COX multivariate analysis in both the discovery and validation cohorts. In the future, we will incorporate more indicators, including those mentioned above, for further validation.

Partial patient with stage II–III CRC received the adjuvant chemotherapy according to the National Comprehensive Cancer Network (NCCN) clinical practice guidelines in the CRC. Adjuvant chemotherapy protocol included FOLFOX, CapeOX, Capecitabine, or 5-FU/leucovorin.

Chemotherapy is generally initiated around 3 weeks after surgery, with a maximum delay of 2 months post-surgery.

Regarding the classification of colon cancer, let me reiterate: in our clinical practice, we mostly classify it as left-sided or right-sided colon cancer. However, in the TCGA database, this important variable is classified as distal vs. proximal, which has posed challenges for our study. We are awaiting further research from our research team to provide clarification on this matter.

The info is valuable and interesting; however, the English language of the manuscript must be rechecked by a native speaker.

Also, the statistics are sophisticated, however, I wish the numbers within the cohorts would be higher to make the analysis more robust.

Answer: Thank you for the comments and recognition from the reviewers. As for the English grammar and vocabulary related issues in this manuscript, our research team is planning to submit it to the AME journal for extensive language editing. Further research on increasing the sample size is currently ongoing in our team.