**Peer Review File**

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**Reviewer A**

1. please tell me if there were any significant differences in patient backgrounds, such as treatment details, between the PCAT Low and High groups.
Reply: Dear Professor, thank you very much for your comments. Your suggestion is indeed very reasonable. After obtaining data from public databases, there was no significant difference in patient background (such as treatment details) between the low and high PCAT groups.

2. In the introduction, I think it would be better to state ctDNA, whose usefulness in colorectal cancer has been reported in recent years.
Reply: Dear Professor, thank you very much for your comments. Your suggestion is indeed very reasonable. Following your valuable suggestion, the role of ctDNA in colorectal cancer has been added in the introduction. Changes in the text: Line 57-61.

3. Please tell me if there is a possibility that PCAT6 can be evaluated by a simple test such as blood sampling as well as CEA and CA19-9.
Reply: Dear Professor, thank you very much for your comments. Your suggestion is indeed very reasonable. At present, in the research stage, it is not possible to evaluate PCAT6 through simple tests such as blood sampling, CEA, and CA19-9.

4. Please tell me if there are any specific new treatment prospects involving PCAT6 mentioned in the discussion.
Reply: Dear Professor, thank you very much for your comments. Your suggestion is indeed very reasonable. The PCAT6 mentioned in the discussion is currently being evaluated for its therapeutic potential in colorectal cancer through a combination of animal experiments, cell experiments, and clinical trials.

**Reviewer B**

1. Methodology Details: Please provide more specific details in the 'RNA extraction and quantitative reverse transcription polymerase chain reaction' section. Include information about normalization and control methods used for better reproducibility.
Reply: Dear Professor, thank you very much for your comments. Your suggestion is indeed very reasonable. Real-Time PCR:
Total RNA was extracted from NCM460 (human normal intestinal epithelial cells), HCT116 (human colon cancer cells), and RKO (human colon adenocarcinoma cells) cell lines using Trizol Reagent (Guangzhou, China). All cell lines were purchased from

Wuhan Punosai Life Science Technology Co., Ltd. in China. Thoroughly remove the culture medium from the 6-well plate, add 800ul-1ml of MagZolTM Reagent to each well of cells, thoroughly blow and mix, leave at room temperature for 5 minutes, and transfer to an enzyme-free EP tube. Add 0.2 times the volume of chloroform, vigorously shake with your hands for 15 seconds, let it stand at room temperature for about 3 minutes, let it layer, and centrifuge at 4 ° C, 12000g for 15 minutes. Suck the clear liquid from the upper layer and transfer it to a new enzyme free 1.5mL EP tube. Add an equal volume of isopropanol and centrifuge at 4 ℃ for 12000 g for 10 minutes. On ice operation, discard the supernatant and add 1mL of 75% ethanol. Invert and mix well. Centrifuge at 4 ℃ for 7500g for 5 minutes. Discard the supernatant and obtain RNA. Use the Thermo Fisher nucleic acid quantitator to measure the concentration and purity of RNA. Then reverse transcribed into cDNA using Vazyme HiScript II Q RT SuperMix. The reverse transcription process is as follows: 5 × Hiscript II qRT SuperMixII (4 μ L) RNase free ddH2O (20 μ L) After mixing with total RNA (1pg-1ug), reverse transcription reaction was performed on a PCR machine according to the following reaction procedure: 50 ℃ (15 min), 85 ℃ (5 s), 4 ℃ (60 min). Real-time fluorescence quantitative PCR was performed using the RT PCR kit (AceQ qPCR SYBR Green Master Mix, Q111-02). The qPCR reaction system is ddH2O (8 μ L) 2 × AceQ QPCR SYBR Green Master Mix (10 μ L) Positive primer (0.4 μ L) Reverse primer (0.4) μ L) And Template DNA (1.2 μ L) Collect fluorescence signals using the Roche LightCycle 480 PCR system instrument. Using GAPDH as the internal reference, finally use $2^{-\Delta\Delta}$ to Calculate the relative expression level of LncRNA PCAT6 using the Ct method. The primer base sequences used (forward/reverse) are as follows:
PCAT6: TGCTTCTACCACCACCTTC/TTCACAGGGGACATCTGACA;
GAPDH: CAGGAGGCATTGCTGATGAT/GAAGGCTGGGGCTCATTT.
The data is represented as three independent experiments.

2. Statistical Analysis: Elaborate on the statistical methods in the ‘Statistical Analysis’ section. Explain the choice of Wilcoxon rank-sum tests, logistic regression, and Cox regression analyses in detail.
Reply: Dear Professor, thank you very much for your comments. Your suggestion is indeed very reasonable. 1. Basic data information: If the variable is of numerical type and the sample size is<=5000, a normality test is performed. When the data satisfies a normal distribution, the mean ± standard deviation of the corresponding variable will be counted. If it does not satisfy a normal distribution, the median (upper and lower quartiles) of the corresponding variable will be counted; If the variable is of numerical type and the sample size is>5000, no normality test will be conducted, and the mean ± standard deviation of the corresponding variable will be calculated.
2. Selection of statistical methods:
If the variable is of numerical type, when the data satisfies a normal distribution and homogeneity of variance test, the method for comparing two groups is a T-test, and the method for comparing three groups is One way ANOVA; When the data satisfies a normal distribution but does not satisfy the homogeneity of variance test, the method for comparing two groups is Welch t-test, and the method for comparing three groups

is Welch one way ANOVA; Not meeting normal distribution, Wilcoxon is the method for comparing two groups, and Kruskal Wallis is the method for comparing three groups. If the variable is classified, the chi-square test is used for inter-group comparison when the data meets the conditions of theoretical frequency>5 and total sample size>=40; When the data meets the condition of 5>theoretical frequency>=1 and total sample size>=40, the continuous corrected chi-square test (Yates' correction) is used for inter-group comparison; When the theoretical frequency of the data is less than 1 or the total sample size is less than 40, Fisher's exact test is used for inter-group comparison.

3. Interpretation of Results: In the 'Results' and 'Discussion' sections, critically evaluate the association between PCAT6 expression and CRC prognosis. Consider alternative explanations and potential confounding factors.
Reply: Dear Professor, thank you very much for your comments. Your suggestion is indeed very reasonable. Strictly evaluate the relationship between PCAT6 expression and CRC prognosis in the Results and Discussion sections. The relationship between PCAT6 expression levels and clinical characteristics in CRC patients in the database. The high expression of PCAT6 is significantly correlated with the N-stage and M-stage pathological staging, lymphoid infiltrating tumor type, and CEA level of the tumor. The findings indicate that CRC patients with increased PCAT6 expression are more likely to develop lymph node metastasis and more advanced diseases. Other potential confounding factors such as tumor T stage, History of colon polyps, and Colon polyps presentation were compared, but there was no significant correlation with PCAT6 expression.

4. Comparative Literature Analysis: Expand the 'Discussion' section with a detailed comparison of existing literature on lncRNAs in CRC.
Reply: Dear Professor, thank you very much for your comments. Your suggestion is indeed very reasonable. Following your valuable suggestion, expand the 'Discussion' section with a detailed comparison of existing literature on lncRNAs in CRC. Changes in the text: Line 208-211.

5. Writing and Structure: Address occasional grammatical errors and awkward phrasings throughout the paper for enhanced readability.
Reply: Dear Professor, thank you very much for your comments. Your suggestion is indeed very reasonable. The manuscript has been carefully edited by experts in technical English.