International Journal of General Medicine

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Predictive Values of Homeobox Gene A-Antisense Transcript 3 (HOXA-AS3), Cystatin 6 (CST6), and Chromobox Homolog 4 (CBX4) Expressions in Cancer Tissues for Recurrence of Early Colon Cancer After Surgery

Xiaopeng Zhu[1,*], Liang Zhao[2,*], Peng Hu[1]

[1]Department of Medical Oncology, Huangshi Central Hospital (Affiliated Hospital of Hubei Polytechnic University), Edong Healthcare Group, Huangshi, Hubei Province, 435000, People's Republic of China; [2]Department of Oncology Surgery, Huangshi Central Hospital (Affiliated Hospital of Hubei Polytechnic University), Edong Healthcare Group, Huangshi, Hubei Province, 435000, People's Republic of China

*These authors contributed equally to this work

Correspondence: Peng Hu, Department of Medical Oncology, Huangshi Central Hospital (Affiliated Hospital of Hubei Polytechnic University), Edong Healthcare Group, Huangshi, 435000, Hubei Province, People's Republic of China, Tel +8615923360125, Email hupenghch@dh-edu.cn

Purpose: We aim to explore the predictive values of homeobox gene A-antisense transcript 3 (HOXA-AS3), cystatin 6 (CST6), and chromobox homolog 4 (CBX4) expressions in cancer tissues for the recurrence of early colon cancer after surgery.

Patients and Methods: A total of 136 patients who received surgery from January 2020 to January 2021 were enrolled and followed up for 24 months to observe the recurrence after surgery, based on which they were assigned into recurrence and non-recurrence groups. All patients underwent a histopathological examination on admission.

Results: The recurrence group had a lower degree of differentiation as well as a higher HOXA-AS3 level and CST6 and CBX4 expression scores than those of the non-recurrence group ($P<0.05$). HOXA-AS3 level, CST6 expression score, and CBX4 expression score were risk factors for the recurrence of early colon cancer after surgery [odds ratio (OR)>1, $P<0.05$]. The receiver operating characteristic curve analysis showed that the areas under the curves of HOXA-AS3 level, CST6 expression score, CBX4 expression score, and their combination for predicting recurrence were 0.909 [95% confidence interval (95% CI): 0.785–1.000], 0.819 (95% CI: 0.690–0.948), 0.794 (95% CI: 0.663–0.926), and 0.942 (95% CI: 0.882–1.000), respectively.

Conclusion: The expressions of HOXA-AS3, CST6, and CBX4 in cancer tissues have close correlations with the recurrence of early colon cancer after surgery and are thus of high predictive values.

Keywords: colon cancer, expression, prediction, recurrence, surgery

Introduction

Colon cancer, a common gastrointestinal tumor, can cause symptoms such as abdominal distension and pain and may lead to the generation of toxins that can be absorbed by human body. Early colon cancer can result in nutrient deficiency, giving rise to manifestations such as emaciation, anemia, weakness, and edema in patients.[1] In addition, it may induce intestinal obstruction and even have distant metastasis if not effectively treated in time, resulting in multi-organ dysfunction and threatening the lives of patients.[2] The detection rate of early colon cancer is on the rise in recent years since increasing numbers of people are receiving regular physical examinations. Early colon cancer is mainly treated by surgery, which can effectively repress tumor progression and relieve clinical symptoms by resecting the diseased tissues, but tumor recurrence still occurs.[3] Recurrent colon cancer is characterized by a higher degree of

Received: 11 September 2023
Accepted: 8 December 2023
Published: 3 January 2024

malignancy, greater treatment difficulty, and poorer prognosis. Therefore, discovering indicators related to colon cancer invasion is important for guiding clinical treatment and predicting prognosis.

Homeobox gene A-antisense transcript 3 (HOXA-AS3) is a novel long non-coding RNA, which belongs to the HOX gene family and can regulate embryonic development and participate in the formation and differentiation of hematopoietic cells at the same time. HOX genes are expressed in various neoplastic diseases and play a key regulatory role in the synthesis and transcription of DNA.[4,5] Long non-coding RNA HOXA-AS3 has a significantly higher expression level in gastric cancer cells and tissues than that in normal gastric cells and adjacent tissues, and its high expression may promote the proliferation, infiltration, and metastasis of gastric cancer cells.[6] The high expression of HOXA terminal transcript-translated RNAs has an association with the prognosis of many malignant neoplastic diseases such as liver cancer and lung cancer.[7] Thus, HOXA-AS3 may have a relationship with the prognosis of patients with colon cancer.

Members of the cystatin family contribute to the progression of various malignant neoplastic diseases such as liver cancer, oral cancer, thyroid cancer, and pancreatic cancer.[8,9] Cystatin 6 (CST6) gene, a member of the cystatin family, is located on chromosome 11q13. CST6 has a reduced expression in osteoblastic breast cancer samples and cells and can inhibit tumor infiltration and bone metastasis.[10] In addition to the inhibitory effect, CST6 also exerts a promoting effect on tumors.[11] For instance, CST6 has a significantly elevated expression in triple-negative breast cancer, which is considered to be a tumor-promoting molecule and has correlations with the low disease-free survival rate and high risk of lymph node metastasis.[12] However, the role of CST6 expression in the recurrence of colon cancer after surgery has seldom been referred.

Chromobox homolog 4 (CBX4) is a member of the CBX family. CBX4 participates in the proliferation, invasion, and metastasis of cancer cells, and tumor growth can be impeded by downregulating CBX4 expression.[13,14] CBX4 expression is significantly higher in breast cancer tissues than that in adjacent tissues, and it is closely associated with high clinical stage and metastasis.[15] In addition, high CBX4 expression can promote the onset and progression of gastric cancer.[16] Prognostic values of CBX family members for colorectal cancer have recently been reported.[17] Nevertheless, the influence of CBX4 on the prognosis of patients with colon cancer still needs clarification.

Thereby motivated, the predictive values of HOXA-AS3, CST6, and CBX4 expressions for the recurrence of early colon cancer after surgery were explored in this study, aiming to render a reference for treatment and prognostic evaluation.

Materials and Methods

Baseline Clinical Data

A total of 136 patients with early colon cancer who underwent surgery in the hospital from January 2020 to January 2021 were included in this study.

Inclusion and Exclusion Criteria

The inclusion criteria were set as follows: (1) patients meeting relevant diagnostic criteria for colon cancer,[18] (2) those definitely diagnosed with primary colon cancer by pathological examination, (3) those with Dukes' stage A colon cancer, (4) those who had undergone surgical treatment, and (5) those who or whose family members signed informed consent. The exclusion criteria involved: (1) patients who had received radiotherapy, chemotherapy, or targeted therapy before surgery, (2) those with contraindications to surgery, (3) those with immune system diseases or hematological disorders, (4) those with severe hepatic or renal insufficiency, (5) those with severe mental illness, or (6) those with other malignant tumors.

Measurement of HOXA-AS3 Expression in Colon Cancer and Adjacent Tissues by Real-Time Quantitative PCR

Following extraction of total RNAs from colon cancer tissues and adjacent tissues (about 5 cm from the lesion) by an RNA extraction kit (Shanghai Beyotime Biotechnology Co., Ltd., China, R0011), the purity and concentration of total RNAs were detected by UV spectrophotometry. Then cDNA was obtained through reverse transcription using

PrimeScript™ II 1st strand cDNA synthesis kit (TaKaRa, Japan, 6210A) and stored in a refrigerator at −20°C. Next, the expression of long non-coding RNA HOXA-AS3 was determined by real-time quantitative PCR. Upstream primer for long non-coding RNA HOXA-AS3: 5'-GAAAGCTGCAACATGCTCCC-3', downstream primer: 5'-TCCATGTCGTCCCAGTTGGT-3' (synthesized by Sangon Biotech (Shanghai) Co., Ltd., China). GAPDH was used as an internal reference. The $2^{-\Delta\Delta CT}$ method was used to calculate the relative expression of target gene.

Measurement of CST6 and CBX4 Expressions in Colon Cancer and Adjacent Tissues by Immunohistochemical Staining

Colon cancer and adjacent tissues were taken and prepared into sections with a thickness of 4 μm, followed by detection of CST6 and CBX4 expressions via immunohistochemical staining (Shanghai Beyotime Biotechnology Co., Ltd., China, P0615). In brief, sections were deparaffinized in xylene (C_8H_{10}) and hydrated in gradients of anhydrous ethanol, 85% ethanol, and 75% ethanol. Afterwards, tissue sections were boiled in citric acid antigen repair buffer (pH 6.0) to repair the antigen, soaked in a 3% hydrogen peroxide solution to block endogenous peroxidase and sealed with 3% bovine serum albumin (BSA). After that, they were incubated with primary antibody against CST6 (Proteintech, USA, 17076-1-AP) and rabbit anti-human CBX4 polyclonal antibody (Elabscience Biotechnology Co., Ltd., China, E-AB-17696) at 4°C overnight, followed by addition of horseradish peroxidase-labeled secondary antibody polymer in appropriate drops and incubation for 50 min in a greenhouse. Thereafter, tissue sections were subjected to color development with diaminobenzidine (DAB), re-staining with hematoxylin, dehydration, and mounting. Then, immunohistochemical staining images were obtained using an automated scanning microscope (Olympus (Beijing) Co., Ltd., China, CKX53) and an image acquisition and analysis system, and five fields of view were selected on each section to count the positive cells. Film reading was completed by a senior pathologist. The staining results were graded according to the intensity of staining as follows : brown (3 points), brownish-yellow (2 points), yellowish (1 point), and colorless (0 point). The percentage of positive cells was classified into >75% (4 points), 51–75% (3 points), 26–50% (2 points), 5–25% (1 point), and <5% (0 point). Final comprehensive expression score = intensity of staining × percentage of positive cells.

Recurrence After Surgery

Patients receiving surgery were followed up by telephone or reexamination in the hospital once every 3–6 months after discharge from the hospital to observe the recurrence of colon cancer after surgery. Patients with recurrence within 24 months after surgery were included in recurrence group, while those without recurrence in the same period were assigned into non-recurrence group.

Observation Indicators

The clinicopathological characteristics of patients with early colon cancer were compared between recurrence group and non-recurrence group, including sex (male or female), age, tumor diameter, pathological type (adenocarcinoma or others), tumor site (right or left hemicolon), degree of differentiation (high, moderate, or poor differentiation), tumor diameter (<3 cm or ≥3 cm), depth of infiltration (T1 or T2), vascular invasion (yes or no), and HOXA-AS3, CST6, and CBX4 expressions.

Statistical Analysis

SPSS 23.0 software was employed for statistical analysis. The measurement data were expressed as (mean ± standard deviation) and subjected to the *t*-test. The count data were expressed as case number (percentage) and subjected to the χ^2 test, and the rank sum test was utilized for rank data analysis. The correlations between continuous and categorical variables were tested by point biserial correlation analysis. A Cox regression analysis was conducted to examine the effects of HOXA-AS3, CST6, and CBX4 on the recurrence of early colon cancer after surgery. Receiver operating characteristic (ROC) curves were plotted to analyze the predictive values of HOXA-AS3, CST6, and CBX4 expressions for the recurrence of early colon cancer after surgery. P<0.05 suggested that the difference was of statistical significance.

Results

The results of 24-month follow-up showed that 12 (8.82%) out of the 136 early colon cancer patients treated with surgery had recurrence after surgery. The cumulative recurrence curve is exhibited in Figure 1.

Among these patients, 87 were males and 49 were females, with an age of 46–68 years old, a body mass index (BMI) of 19–29 kg/m^2, and a tumor diameter of 1.9–5.8 cm. The mean age, BMI, and tumor diameter were (53.68±4.63) years old, (25.86 ±2.13) kg/m^2, and (3.52±0.76) cm, respectively. The degree of differentiation was lower in recurrence group than that in non-recurrence group (P<0.05), whereas the sex, age, tumor diameter, pathological type, tumor site, tumor diameter, depth of infiltration, and vascular invasion were statistically identical between the two groups (P>0.05) (Table 1).

The recurrence group had higher HOXA-AS3 level, CST6 expression score, and CBX4 expression score [3.28±1.13, (5.62±1.37) point and (5.81±1.42) point] than those of the non-recurrence group [1.79±0.42, (4.07±1.14) point and (4.19 ±1.21) point] (P<0.001). The representative immunohistochemical staining images of CST6 and CBX4 expressions are shown in Figure 2A, and the statistical chart for HOXA-AS3, CST6, and CBX4 is exhibited in Figure 2B.

According to point biserial correlation analysis, positive correlations were found between HOXA-AS3, CST6, and CBX4 expressions and the recurrence of early colon cancer after surgery (r>0, P<0.05). Besides, HOXA-AS3, CST6, and CBX4 expressions were positively correlated with each other (r>0, P<0.05) (Table 2).

Cox regression analysis was carried out with HOXA-AS3 level, CST6 expression score, and CBX4 expression score as independent variables (continuous variables) and the recurrence of early colon cancer after surgery as the dependent variable (1=recurrence, 0=no recurrence). It was found that HOXA-AS3 level, CST6 expression score, and CBX4 expression score were risk factors for the recurrence of early colon cancer after surgery [odds ratio (OR)>1, P<0.05] (Table 3).

According to the ROC curve plotted using HOXA-AS3 level (Figure 3), CST6 expression score and CBX4 expression score as the test variables and the recurrence of early colon cancer after surgery as the status variable (1=recurrence, 0=no recurrence), the area under the curve (AUC) of HOXA-AS3 level, CST6 expression score, CBX4 expression score, and the combination of the three indicators for predicting the recurrence of early colon cancer after surgery was 0.909 (95% CI: 0.785–1.000), 0.819 (95% CI: 0.690–0.948), 0.794 (95% CI: 0.663–0.926), and 0.942 (95% CI: 0.882–1.000), respectively. The relevant parameters are shown in Table 4.

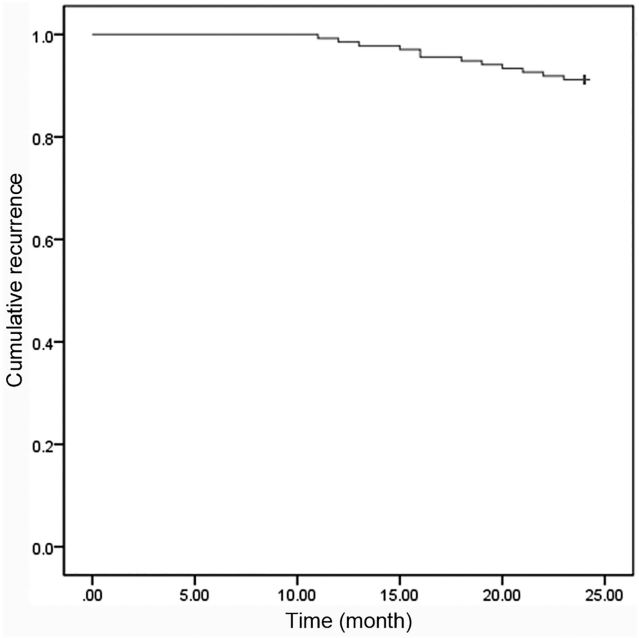

Figure 1 Cumulative recurrence curve.

Table 1 Clinicopathological Characteristics in Recurrence and Non-Recurrence Groups

Clinicopathological Characteristics		Recurrence Group (n=12)	Non-Recurrence Group (n=124)	Statistic value	P
Sex [n (%)]	Male (n=87)	7 (58.33)	80 (64.52)	χ^2=0.012	0.912
	Female (n=49)	5 (41.67)	44 (35.48)		
Age ($\bar{x} \pm s$, year)		54.16±2.79	53.63±4.57	t=0.394	0.694
Tumor diameter ($\bar{x} \pm s$, cm)		3.71±0.69	3.48±0.75	t=1.021	0.309
Pathological type [n (%)]	Adenocarcinoma (n=113)	10 (83.33)	103 (83.06)	χ^2=0.144	0.704
	Others (n=23)	2 (16.67)	21 (16.94)		
Tumor site [n (%)]	Right hemicolon (n=60)	5 (41.67)	55 (44.35)	χ^2=0.032	0.858
	Left hemicolon (n=76)	7 (58.33)	69 (55.65)		
Degree of differentiation [n (%)]	High differentiation (n=91)	4 (33.33)	87 (70.16)	Z=2.642	0.008
	Moderate differentiation (n=31)	5 (41.67)	26 (20.97)		
	Poor differentiation (n=14)	3 (25.00)	11 (8.87)		
Tumor diameter [n (%)]	<3 cm (n=43)	4 (33.33)	39 (31.45)	χ^2=0.037	0.848
	≥3 cm (n=93)	8 (66.67)	85 (68.55)		
Depth of infiltration [n (%)]	T1 (n=79)	6 (50.00)	73 (58.87)	χ^2=0.354	0.552
	T2 (n=57)	6 (50.00)	51 (41.13)		
Vascular invasion [n (%)]	Yes (n=10)	1 (8.33)	9 (7.26)	χ^2=0.050	0.823
	No (n=126)	11 (91.67)	115 (92.74)		

Discussion

There are approximately 1.2 million new cases of colon cancer each year on a global scale, and the prognosis of patients with colon cancer is still far from satisfactory despite recent developments in medical equipment and technologies.[19] Therefore, suppressing the growth, infiltration, and migration of colon cancer cells is a crucial approach for improving the prognosis of patients. Given that determining the prognosis based on tumor stage alone may have bias, discovering novel reasonable biomarkers is of great clinical significance.

Chen et al reported that HOXA-AS3 was related to tumor recurrence in colon adenocarcinoma.[20] In this study, the HOXA-AS3 level in the recurrence group was significantly higher than that in the non-recurrence group, indicating that HOXA-AS3 may be a risk factor for the recurrence of colon cancer after surgery. Additionally, the CST6 expression rose in colon cancer tissues and that the CST6 expression score was significantly higher in the recurrence group, suggesting

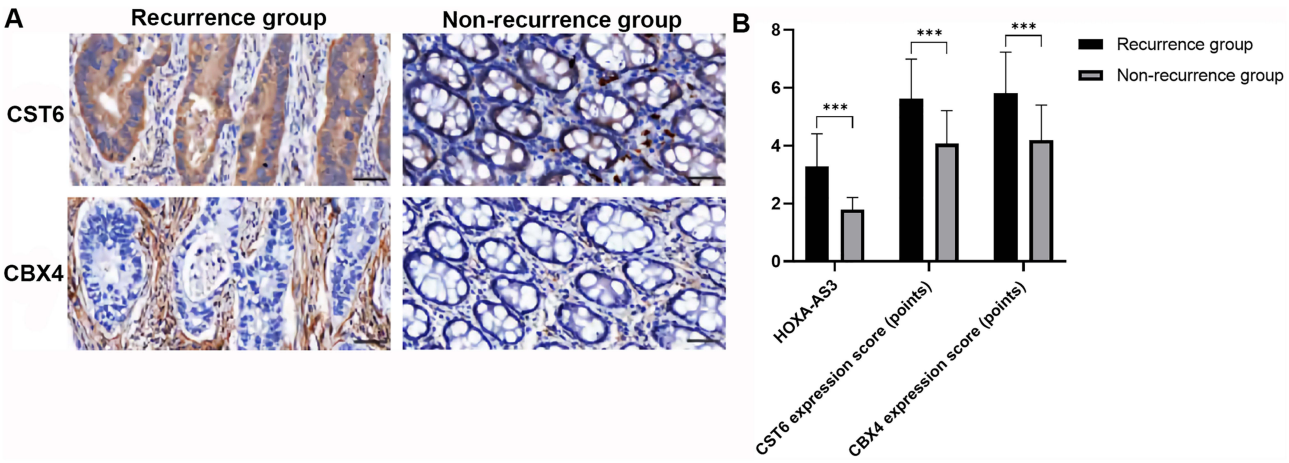

Figure 2 (**A**) Representative immunohistochemical staining images of CST6 and CBX4 expressions; (**B**) statistical chart for HOXA-AS3, CST6 and CBX4. ***P<0.001.

Table 2 Correlations Between HOXA-AS3, CST6, and CBX4 Expressions and Their Correlations with Recurrence of Early Colon Cancer After Surgery

	Group	HOXA-AS3 Level	CST6 Expression Score	CBX4 Expression Score
Group	–	0.639/<0.001	0.355/<0.001	0.352/<0.001
HOXA-AS3	0.639/<0.001	–	0.322/<0.001	0.210/0.014
CST6 expression score	0.355/<0.001	0.322/<0.001	–	0.136/0.114
CBX4 expression score	0.352/<0.001	0.210/0.014	0.136/0.114	–

Table 3 Effects of HOXA-AS3, CST6, and CBX4 on Recurrence of Early Colon Cancer After Surgery

Indicator	β	Standard Deviation	Wald χ^2	P	OR	95% CI
HOXA-AS3 level	2.238	0.515	18.856	<0.001	9.372	3.413–25.734
CST6 expression score	1.646	0.443	13.812	<0.001	5.184	2.176–12.346
CBX4 expression score	1.547	0.405	14.561	<0.001	4.697	2.122–10.396

that CST6 may act as a cancer-promoting gene in colon cancer and a potential molecular marker for predicting the prognosis of patients. The results are consistent with those of Wu et al.[21]

In this study, the CBX4 expression score was significantly increased in the recurrence group in comparison with that in the non-recurrence group, indicating that high CBX4 expression may be associated with the poor prognosis of patients with colon cancer. Similarly, Wei et al reported that CBX4 was a potential predictor for the poor prognosis of patients with colon cancer.[22] Moreover, CBX4 expression was positively correlated with the recurrence of early colon cancer after surgery. Possibly, high CBX4 expression may increase the malignancy and necrosis of tumors by enhancing the

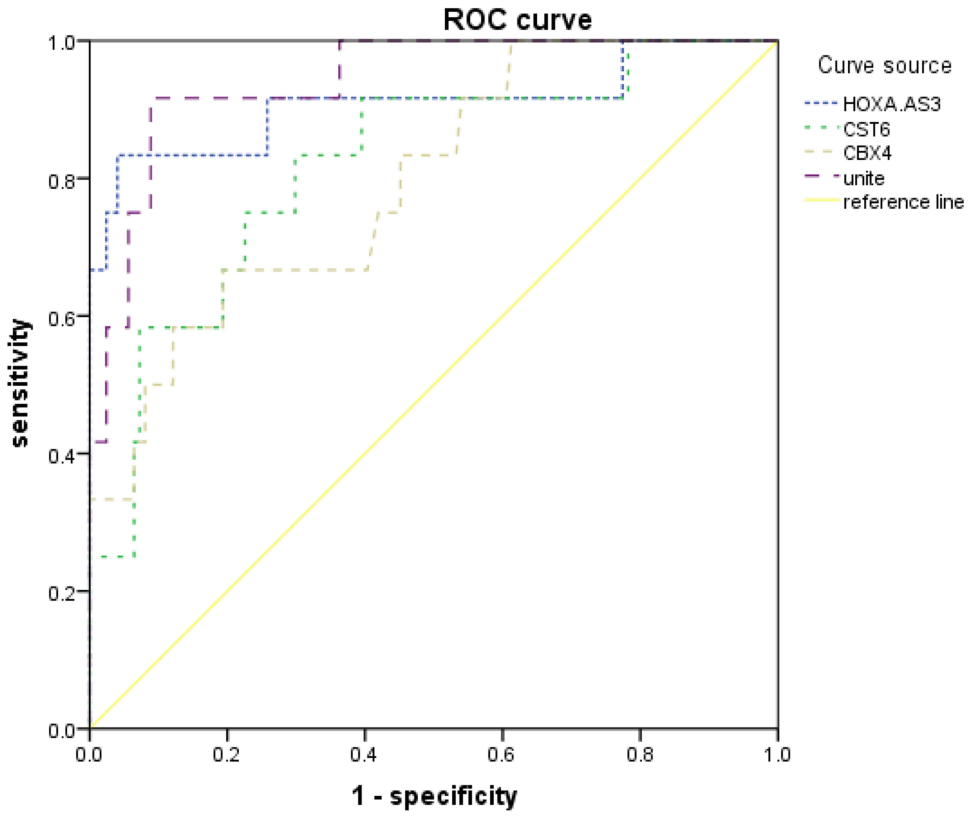

Figure 3 ROC curves of HOXA-AS3, CST6, and CBX4 expressions in predicting recurrence of early colon cancer after surgery.

Table 4 Predictive Values of HOXA-AS3, CST6, and CBX4 Expressions for Recurrence of Early Colon Cancer After Surgery

Variable	AUC	Standard Deviation	*P*	95% CI	Cut-Off	Sensitivity	Specificity	Youden Index
HOXA-AS3 level	0.909	0.063	<0.001	0.785–1.000	2.075	0.917	0.742	0.659
CST6 expression score	0.819	0.066	<0.001	0.690–0.948	4.635 points	0.833	0.702	0.535
CBX4 expression score	0.794	0.067	0.001	0.663–0.926	4.480 points	0.750	0.581	0.331
Combination	0.942	0.030	<0.001	0.882–1.000	–	0.917	0.637	0.554

transcriptional activity of hypoxia-inducible factor-1α through promoting the SUMOylation,[23] thereby affecting the prognosis of patients.

Furthermore, the AUCs of HOXA-AS3 level, CST6 expression score, and CBX4 expression score for predicting the recurrence of early-stage colon cancer after surgery were all >0.7, suggesting moderate predictive values of these three indicators. Their predictive value was highest when the cut-off values were 2.075, 4.635, and 4.480, respectively. The combination of the three indicators had a higher predictive value. These results suggest that particular attention can be paid to the above indicators to assess the recurrence risk of colon cancer after surgery.

Conclusion

In conclusion, the expressions of HOXA-AS3, CST6, and CBX4 in cancer tissues are closely associated with the recurrence of early colon cancer after surgery, and they have certain value in predicting the recurrence in patients after surgery. Clinically, the risk of recurrence in patients with early colon cancer after surgery can be predicted by detecting the expression of these indicators, and the prognosis of patients can be improved by taking early preventive interventions. Nevertheless, this study is limited. The sample size is small, and the findings are obtained from a single medical center. Additionally, this conclusion is not validated in an independent patient cohort. Further studies are ongoing in our group.

Ethics Statement

Research involving human subjects complied with all relevant national regulations and institutional policies and is in accordance with the tenets of the Helsinki Declaration (as revised in 2013) and has been approved by Huangshi Central Hospital (Affiliated Hospital of Hubei Polytechnic University; approval No. HCH202001005). Written informed consent was obtained from all individuals included in this study.

Funding

There is no funding to report.

Disclosure

The authors declare no conflict of interest.

References

1. Scott N, West NP, Cairns A, Rotimi O. Is medullary carcinoma of the colon underdiagnosed? An audit of poorly differentiated colorectal carcinomas in a large national health service teaching hospital. *Histopathology*. 2021;78(7):963–969. doi:10.1111/his.14310
2. Schulc K, Nagy ZT, Kamp S, et al. Modular reorganization of signaling networks during the development of colon adenoma and carcinoma. *J Phys Chem B*. 2021;125(7):1716–1726. doi:10.1021/acs.jpcb.0c09307
3. Lee M, Kudose S, Del Portillo A, et al. Invasive carcinoma versus pseudoinvasion: interobserver variability in the assessment of left-sided colorectal polypectomies. *J Clin Pathol*. 2022;75(9):593–597. doi:10.1136/jclinpath-2021-207406
4. Qu F, Zhu B, Hu YL, Mao QS, Feng Y. LncRNA HOXA-AS3 promotes gastric cancer progression by regulating miR-29a-3p/LTβR and activating NF-κB signaling. *Cancer Cell Int*. 2021;21(1):118. doi:10.1186/s12935-021-01827-w
5. Li L, Wang Y, Song G, Zhang X, Gao S, Liu H. HOX cluster-embedded antisense long non-coding RNAs in lung cancer. *Cancer Lett*. 2019;450:14–21. doi:10.1016/j.canlet.2019.02.036
6. Chen D, Xie S, Wu Y, et al. Reduction of bladder cancer chemosensitivity induced by the effect of HOXA-AS3 as a ceRNA for miR-455-5p that upregulates Notch1. *Front Oncol*. 2021;10:572672. doi:10.3389/fonc.2020.572672

7. Zhang H, Liu Y, Yan L, et al. Long noncoding RNA Hoxaas3 contributes to hypoxia-induced pulmonary artery smooth muscle cell proliferation. *Cardiovasc Res*. 2019;115(3):647–657. doi:10.1093/cvr/cvy250
8. Li X, Liang Y, Lian C, et al. CST6 protein and peptides inhibit breast cancer bone metastasis by suppressing CTSB activity and osteoclastogenesis. *Theranostics*. 2021;11(20):9821–9832. doi:10.7150/thno.62187
9. Xie Q, Liu L, Chen X, et al. Identification of Cysteine Protease Inhibitor CST2 as a Potential Biomarker for Colorectal Cancer. *J Cancer*. 2021;12(17):5144–5152. doi:10.7150/jca.53983
10. Theivakadadcham VS, Rosonina E. Ethanol stress stimulates sumoylation of transcription factor Cst6 which restricts expression of its target genes. *bioRxiv*. 2019. doi:10.1101/758607
11. Lalmanach G, Kasabova-Arjomand M, Lecaille F, Saidi A. Cystatin M/E (cystatin 6): a janus-faced cysteine protease inhibitor with both tumor-suppressing and tumor-promoting functions. *Cancers*. 2021;13(8):1877. doi:10.3390/cancers13081877
12. Li Q, Zheng ZC, Ni CJ, et al. Correlation of cystatin E/M with clinicopathological features and prognosis in triple-negative breast cancer. *Ann Clin Lab Sci*. 2018;48(1):40–44. PMID: 29530995.
13. Wang Z, Fang Z, Chen G, et al. Chromobox 4 facilitates tumorigenesis of lung adenocarcinoma through the Wnt/β-catenin pathway. *Neoplasia*. 2021;23(2):222–233. doi:10.1016/j.neo.2020.12.005
14. Ren X, Hu B, Song M, et al. Maintenance of nucleolar homeostasis by CBX4 alleviates senescence and osteoarthritis. *Cell Rep*. 2019;26(13):3643–3656.e7. doi:10.1016/j.celrep.2019.02.088
15. Sanyal S, Mondal P, Sen S, Sengupta Bandyopadhyay S, Das C. SUMO E3 ligase CBX4 regulates hTERT-mediated transcription of CDH1 and promotes breast cancer cell migration and invasion. *Biochem J*. 2020;477(19):3803–3818. doi:10.1042/BCJ20200359
16. Yang Z, OuYang X, Zheng L, Dai L, Luo W. Long intergenic noncoding RNA00265 promotes proliferation of gastric cancer via the microRNA-144-3p/Chromobox 4 axis. *Bioengineered*. 2021;12(1):1012–1025. doi:10.1080/21655979.2021.1876320
17. Li Q, Pan Y, Cao Z, Zhao S. Comprehensive analysis of prognostic value and immune infiltration of chromobox family members in colorectal cancer. *Front Oncol*. 2020;10:582667. doi:10.3389/fonc.2020.582667
18. Benson AB, Venook AP, Al-Hawary MM, et al. NCCN guidelines insights: colon cancer, Version 2.2018. *J Natl Compr Canc Netw*. 2018;16(4):359–369. doi:10.6004/jnccn.2018.0021
19. Feng H, Lyu Z, Zheng J, et al. Association of tumor size with prognosis in colon cancer: a Surveillance, Epidemiology, and End Results (SEER) database analysis. *Surgery*. 2021;169(5):1116–1123. doi:10.1016/j.surg.2020.11.011
20. Chen F, Li Z, Deng C, Yan H. Integration analysis for novel lncRNA markers predicting tumor recurrence in human colon adenocarcinoma. *J Transl Med*. 2019;17(1):299. doi:10.1186/s12967-019-2049-2
21. Wu DJ, Xu AJ, Yu B. CST6在结肠癌中的表达及与肿瘤血管生成的关系 [Expression of CST6 in colon cancer and its relationship with tumor angiogenesis]. *Fudan Univ J Med Sci*. 2022;49(05):642–648, 664. Chinese. doi:10.3969/j.issn.1672-8467.2022.05.002
22. Wei W, Zhao W, Zhang Y. CBX4 provides an alternate mode of colon cancer development via potential influences on circadian rhythm and immune infiltration. *Front Cell Dev Biol*. 2021;9:669254. doi:10.3389/fcell.2021.669254
23. Li J, Xu Y, Long XD, et al. Cbx4 governs HIF-1α to potentiate angiogenesis of hepatocellular carcinoma by its SUMO E3 ligase activity. *Cancer Cell*. 2014;25(1):118–131. doi:10.1016/j.ccr.2013.12.008