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ORIGINAL ARTICLE

DNA topoisomerase II alpha: a favorable prognostic factor in colorectal caner

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Abstract
Purpose There is a lack of study concerning expression of Topoisomerase IIα (Topo IIα) and long-term results in colorectal cancer patients. We aimed to investigate the relationship between expression of Topo IIα and clinicopathological parameters including overall survival in colorectal cancer.
Methods Paraffin-fixed specimens from a large prospective cohort of colorectal cancer patients who had been followed up for 4 years were assayed immunohistochemically.
Results Of 490 colorectal cancer patients accessible for Topo IIα expression, expression of Topo IIα was scored as (−) in 4 (0.8%) patients, (+) in 41 (8.4%) patients, (++) in 396 (80.8%) patients, and (+++) in 49 (10.0%) patients. Overexpression of Topo IIα was found to be related with lower T stage (p=0.042), lower N stage (p=0.038), and a lower incidence of recurrence with nearly significance (p=0.053). Kaplan–Meier analyses showed that overexpression of Topo IIα was related with prolonged overall survival (p=0.022) and disease-free survival (p=0.036). Multivariate analyses showed that elevated serum CEA (p<0.001), elevated serum CA199 (p=0.002), poor differentiation (p=0.001), advanced Dukes stage (p<0.001), and lower expression of Topo IIα (p=0.017) were independent predictive factors for poor prognosis.
Conclusions Topo IIα expression is a valuable prognostic indicator for colorectal cancer and would be useful in treatment selection for early colorectal cancer and malignant colorectal polyps resected under endoscopy, especially when it is used in combination with serum CEA, CA199, and differentiation.

Keywords Topoisomerase IIα · Colorectal cancer · Immunohistochemistry · Recurrence · Overall survival

Introduction

Colorectal cancer is one of the leading causes of cancer death in the world. At present, tumor stage-related factors (such as TNM stage, Dukes stage, invasion depth, and lymph node status), tumor differentiation, vascular invasion, lymphatic invasion, and status of resection margins constitute the accepted prognostic indicators of surgically treated colorectal cancer [1, 2]. However, tumor stage-related factors sometimes are unavailable, such as in early colorectal cancer detected by colonoscopy or malignant colorectal polyps resected under endoscopy. For these colorectal cancer patients, prognostic factors are extremely limited. Biological markers can be available even in a small amount of tumor tissue, such as in biopsy specimens and endoscopically resected specimens. It is therefore important to identify potential biological markers that can predict long-term survival of colorectal cancer patients [3]. Extensive research into the biology of colorectal cancer has identified a plethora of molecular markers reputed to provide prognostic information [4], but few biological markers are justified for prognosis prediction and treatment

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selection in colorectal cancer [5, 6]. DNA topoisomerase II (Topo II) is a nuclear enzyme involved in the regulation of topological state of DNA [7]. Topo II catalyzes the transient breaking and the subsequent rejoining of the double-strand DNA and acts as an ATP-driven clamp that captures one DNA segment and transports it through the enzyme-bridged break in the second DNA duplex [8]. Topo II unwind and uncoil supercoiled DNA by transiently breaking and rejoining double strands of the DNA duplex that occurs during cellular vital processes, such as transcription, replication, chromosomal segregation, chromosome condensation, and so on [7–10]. In addition to the fundamental role of the Topo II in cell growth and development, it is also the target of several anticancer drugs such as anthracyclines, amsacrine, and so on [8]. Topo II exists as two highly homologous isoforms, Topo IIα and Topo IIβ, which differ in their production during the cell cycle [11]. The expression of Topo IIα is higher in rapidly proliferating cells and is cell cycle dependent [9]. It was noticed in our clinical practice that Topo IIα overexpression tended to be associated with improved prognosis. In order to elucidate the clinical significance of Topo IIα expression in colorectal cancer, we enforced this study to investigate the expression of Topo IIα in 490 colorectal cancer patients and its association with clinicopathologic parameters and long-term prognosis.

Materials and methods

The study protocol was approved by the ethics committee of the Changhai Hospital of Second Military Medical University, and the research project was performed in accordance with the ethical standards of the 1964 Declaration of Helsinki. All patients signed the informed consent form. All tumors were diagnosed at the Department of Pathology of Changhai Hospital of the Second Military Medical University in Shanghai, China. Pathology was classified according to the WHO classification of colorectal tumors, including Duke's staging, AJCC/UICC TNM stage, tumor gross type and differentiation [12].

Immunohistochemistry of Topo IIα

Expression of Topo IIα in tumor tissues from the 490 patients was examined by immunohistochemistry. All of the 490 patients were included in our colorectal cancer database with complete follow-up information and clinicopathological factors. Immunohistochemistry was performed using DAB-based staining technique (Dako ChemMate™Envision™ Kit, Denmark). Paraffin-embedded sections were fixed with 10% neutral buffered formalin. After deparaffinization and rehydration, antigen retrieval was done with citrate buffer solution (0.1 mol/l, pH=6.0) by pressure cooker. The tissue sections were preincubated with 3% hydrogen peroxide and 4% normal goat serum to block nonspecific reactions. A monoclonal antibody to Topo IIα (DAKO, Denmark; dilution 1:40) was incubated on the slides for 60 min in a humidified chamber. HRP-conjugated secondary antibody was applied neat for 30 min. The sections were stained with DAB and counterstained with Mayer's hematoxylin, washed again, dehydrated in alcohol, cleared in xylene, mounted with Pertex mounting medium, and coverslipped.

Staining label classification standards

Topo IIα labeling indices were defined as the percentage of positive nuclei by counting 1,000 cells in high-power fields (×200). Labeling indices of Topo IIα were expressed semiquantitatively by assigning tumors to one of four categories: negative (−), 0~10% cells stained; weakly positive (+), 11~30% cells stained; moderately positive (++), 31~70% cells stained; and strongly positive (+++), 71~100% cells stained. Negative control sections were processed by omitting the primary antibody. Positive control sections were processed from colorectal cancer previously shown to have expressed high levels of the proteins examined.

Statistical analysis

Associations between expression of Topo IIα and clinicopathological variables were analyzed by nonparametric analysis. The Kaplan–Meier method was used to estimate survival. Survival differences were analyzed by logrank test. Multivariate regression analysis was employed with the multivariate Cox proportional hazard model, using stepwise regression (forward: LR). Only factors that had statistical significance ($p<0.05$) in univariate regression analysis were included in multiple regression analysis. The enter limit and remove limit were $p=0.05$ and $p=0.10$, respectively. All tests were carried out using the SPSS 17.0. All p values were two sided, and p values less than 0.05 were considered as statistically significant.

Results

The resected specimens from the 490 colorectal cancer patients who underwent resection in the Colorectal Surgery Department of Changhai Hospital between January 2006 and January 2008 were collected. The patients ranged in age from 27 to 93 years (median 62 years). Of the 490 cases, 301 were located in the rectum and 189 in the colon; 85 cases (17.3%) were classified as T1, 178 (36.3%) as T2, 198 (40.4%) as T3, 24 (4.9%) as T4, and 5 (1.0%) as

unknown. Simultaneous lymph node metastases were found in 218 cases (44.5%), and simultaneous distant metastases in 24 cases (4.9%). Patients were followed up for a median of 30 months (range 6–46 months). Eighty-four patients were lost during the follow-up period. The clinicopathological data were summarized in Table 1.

Relationships between expression of Topo IIα and clinicopathologic characteristics (Table 1)

Nuclear staining of Topo IIα was scored as (−), (+), (++), and (+++) in 4 (0.8%), 41 (8.4%), 396 (80.8%), and 49 (10.0%) cases, respectively. Different staining levels of Topo IIα in colorectal cancer cells were showed in Fig. 1. Overexpression of Topo IIα was related with lower T stage (p=0.042), lower N stage (p=0.038), and a lower incidence of recurrence with nearly statistical significance (p=0.053, Table 1). Spearman correlation coefficients between Topo IIα expression and T stage, N stage, and recurrence were −0.109 (p=0.017), −0.095 (p=0.039), and −0.104 (p=0.021), respectively. No significant association was observed between expression of Topo IIα and gender, age, tumor position, TNM stage, Dukes stage, serum CEA, serum CA199, differentiation, and gross appearance.

Relationships between Topo IIα expression and overall survival and disease-free survival (Fig. 2)

Kaplan–Meier analyses showed that Topo IIα expression was positively related with cumulative overall survival (Fig. 2a, logrank test, p=0.022) and cumulative disease-free survival (Fig. 2b, p=0.036).

Table 1 Relationship of Topo IIα expression and clinicopathologic factors in colorectal cancer patients

Variables		Topo IIα					
		Case	(−)	(+)	(++)	(+++)	p value
Gender[a]	Male	283	2	21	237	23	0.625
	Female	207	2	20	159	26	
Age[a]	≤60 years	219	3	22	174	20	0.156
	>60 years	271	1	19	222	29	
Position[a]	Colon	189	2	20	150	17	0.167
	Rectum	301	2	21	246	32	
T stage[b]	T1	16	0	0	13	3	0.042
	T2	91	0	6	75	10	
	T3	360	4	29	291	36	
	T4	18	0	4	14	0	
N stage[b]	N0	255	3	15	208	29	0.038
	N1	133	0	10	109	14	
	N2	85	1	12	67	5	
TNM[b]	1	85	0	1	75	9	0.213
	2	178	3	15	140	20	
	3	198	1	22	157	18	
	4	24	0	1	21	2	
Dukes[b]	A	16	0	2	13	1	0.250
	B	243	3	15	199	26	
	C	181	1	15	147	18	
	D	50	0	9	37	4	
CEA[a]	(−)	282	2	28	220	32	0.665
	(+)	95	1	6	78	10	
CA199[a]	(−)	292	2	26	230	34	0.426
	(+)	84	1	8	68	7	
Gross type[a]	Mass	105	1	9	80	15	0.317
	Ulcerative	379	3	30	313	33	
Differentiation[b]	Well	25	0	1	24	0	0.115
	Moderate	378	2	28	306	42	
	Poor	84	2	11	64	7	
Recurrence[a]	No	378	3	27	307	41	0.053
	Yes	112	1	14	89	8	

[a]Using the Mann–Whitney U test

[b]Using the Kruskal–Wallis H test

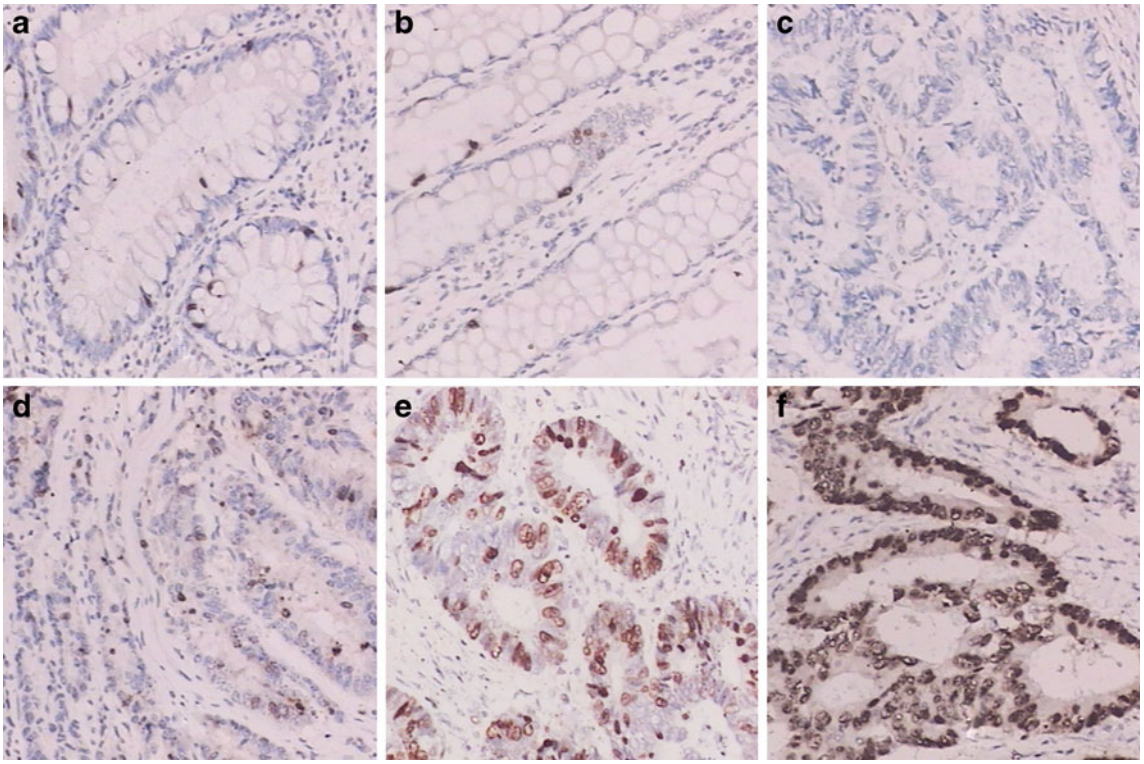

Fig. 1 **a**, **b** Topo IIα expression in normal colorectal mucosa (×200) showed scarce colorectal epithelial cells stained. **c**–**f** Topo IIα expression in colorectal cancer tissue (×200): **c** Topo IIα (−), about 0% colorectal cancer cells stained; **d** Topo IIα (+), about 15% colorectal cancer cells stained; **e** Topo IIα (++), about 65% colorectal cancer cells stained; **f** Topo IIα (+++), about 95% colorectal cancer cells stained

Univariate and multivariate Cox proportional hazard analyses of prognostic factors in colorectal cancer

Univariate analyses (Table 2) identified elevated serum CEA ($p<0.001$), elevated serum CA199 ($p<0.001$), poor differentiation ($p<0.001$), advanced Dukes stage ($p<0.001$), positive lymph node ($p<0.001$), lower expression of Topo IIα ($p=0.013$), advanced TNM stage ($p<0.001$), advanced T stage ($p<0.001$), and ulcerative gross type ($p=0.020$) as predictive factors for shorter overall survival. Multivariate analyses identified elevated serum CEA ($p<0.001$), elevated serum CA199 ($p=0.002$), poor differentiation ($p=0.001$), advanced Dukes stage ($p<0.001$), and lower expression of Topo IIα ($p=0.017$) as independent predictive factors for poor prognosis (Table 3). Compared with patients with Topo IIα (−) expression, patients with Topo IIα (+) ($p=0.038$), Topo IIα (++) ($p=0.007$), and Topo IIα (+++) ($p=0.002$) were associated with prolonged overall survival.

Discussion

The result of this study indicated that Topo IIα protein expression was a valuable prognostic marker for colorectal cancer patients. To the best of our knowledge, this was the first report on the relationship between the overexpression of Topo IIα protein and improved long-term survival in colorectal cancer. This finding validated the value of Topo IIα immunostaining examination in colorectal cancer. High levels of Topo IIα protein expression had been identified in several studies [13, 14]. However, our finding revealed a higher positive expression rate (99.18%) of Topo IIα in colorectal cancer than the positive rate of 69% and 86.7% in previous studies [13, 14]. The discrepancy between our and other studies may be attributable to the different immunohistochemical methods and staining label classification standards used. We used the EnVision immunostaining method, which was much more sensitive than the traditional method such as S-P method or ABC method. The relationship of Topo IIα protein expression and clinicopathologic factors was also investigated in the literature. Ou et al. [14] reported that Topo IIα expression in colorectal tumor tissue was significantly higher than that in normal tissue, and related with TNM stage, Dukes stage, differentiation, and lymph node involvement. They also found that cases with well differentiation, lower TNM stage, lower Dukes stage, and absence of lymph node involvement were associated with higher expression of Topo IIα, seemingly suggesting that overexpression of

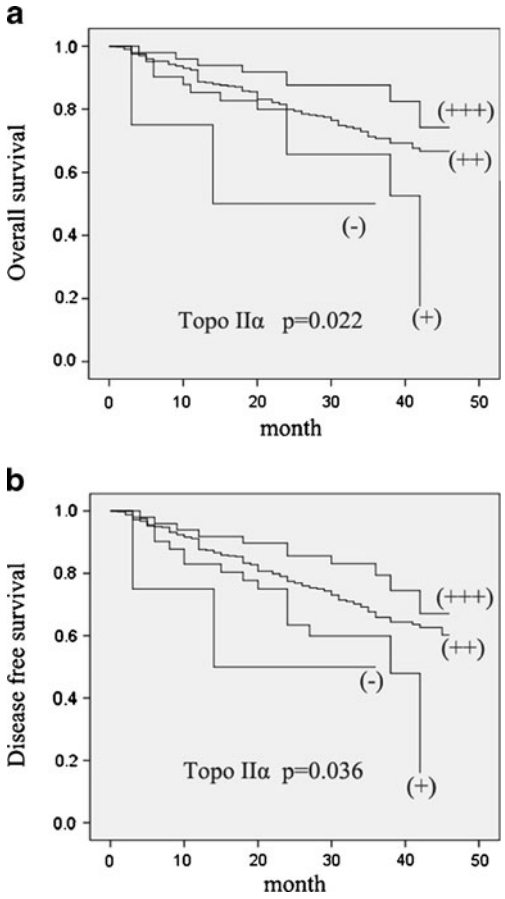

Fig. 2 Kaplan–Meier survival curve in colorectal cancer patients according to the expression level of Topo IIα. **a** Overall survival, p=0.022; **b** disease-free survival, p=0.036

Topo IIα was related with improved prognosis, though survival-related factors were unavailable in their report.

Table 2 Univariate Cox analysis of prognostic factors in CRC

Factors	Univariate	
	HR (95% CI)	p value
CEA (<10 vs. ≥10 ng/ml)	4.54 (3.03–6.79)	<0.001
CA199 (<37 vs. ≥37 U/ml)	3.89 (2.60–5.84)	<0.001
Differentiation (well or moderate vs. poor or mucinous)	2.24 (1.53–3.28)	<0.001
Topo IIα (−~+ vs. ++~+++)	0.53 (0.32–0.88)	0.013
Type (mass vs. ulcerative)	1.79 (1.10–2.91)	0.020
T stage (T1~T2 vs. T3~T4)	3.14 (1.74–5.69)	<0.001
Lymph node (negative vs. positive)	2.54 (1.77–3.66)	<0.001
TNM (I~II vs. III~IV)	2.72 (1.90–3.90)	<0.001
Dukes (A~B vs. C~D)	3.08 (2.13–4.44)	<0.001
P53 (−~+ vs. ++~+++)	0.91 (0.65–1.28)	0.593
Ki67 (−~+ vs. ++~+++)	0.83 (0.37–1.88)	0.652
Age (<60 vs. ≥60 years)	1.14 (0.81–1.62)	0.448
Sex (male vs. female)	1.03 (0.73–1.46)	0.855

Table 3 Multivariate Cox proportional hazard analysis of prognostic factors in CRC

Prognostic factors	HR (95% CI)	p value
Topo IIα	–	0.017
Topo IIα (+)[a]	0.19 (0.04–0.91)	0.038
Topo IIα (++)[a]	0.13 (0.03–0.58)	0.007
Topo IIα (+++)[a]	0.08 (0.02–0.41)	0.002
Dukes (A, B vs. C, D)	2.74 (1.73–4.35)	<0.001
CEA (<10 vs. ≥10 ng/ml)	3.07 (1.95–4.81)	<0.001
CA199 (<37 vs. ≥37 U/ml)	2.01 (1.28–3.15)	0.002
Differentiation (well or moderate vs. poor or mucinous)	2.13 (1.36–3.33)	0.001
Type (mass vs. ulcerative)	1.66 (0.92–2.99)	0.093
T stage (T1~T2 vs. T3~T4)	1.50 (0.66–3.39)	0.335
Lymph node (negative vs. positive)	0.41 (0.17–1.02)	0.054
TNM (I~II vs. II~IV)	2.51 (0.66–9.55)	0.176

[a] Compared with the Topo IIα (–) group

However, Coss et al. [13] reported a contrary result in their study that higher expression of Topo IIα was associated with advanced tumor stage and poor differentiation, suggesting that Topo IIα may play a role in colorectal cancer progression. Coss et al. [13] used chi-square analysis to analyze the relationship between Topo IIα expression and clinicopathological variables. Since the expression of Topo IIα was recorded in the form of ranked data, nonparametric analysis was superior to chi-square analysis in Coss' study. A further review of Coss' study would see that "the expression of Topo IIα varied in different staged cancers and variously differentiated cancer" by chi-square analysis, whereas it was unable to tell whose expression level was higher by nonparametric analysis. Our finding showed that the overexpression of Topo IIα was associated with lower T stage, lower N stage, and a lower incidence of recurrence as well (p=0.053), suggesting that Topo IIα is frequently overexpressed in colorectal cancer and may serve as a predictive marker for prognosis of colorectal cancer patients. To verify this assumption, we enforced Kaplan–Meier analysis (Fig.1) and confirmed that increased expression of Topo IIα was associated with prolonged overall survival and disease-free survival. To further confirm its prognostic value, we employed univariate and multivariate analyses to clarify its contribution to overall survival. The result of univariate analysis showed that higher expression of Topo IIα was significantly correlated with improved prognosis. The result of multivariate Cox proportional hazard analysis also confirmed that Topo IIα expression was independent prognostic factor (Table 3). Compared with patients with Topo IIα (−) expression, patients with Topo IIα (+), Topo IIα (++), and Topo IIα (+++) were associated with prolonged overall survival,

confirming that the expression level of Topo IIα was positively related with improved prognosis. For these reasons, the expression of Topo IIα is helpful in evaluating the prognosis of colorectal cancer patients and choosing the optimized personalized treatment for colorectal cancer patients. The use of a single tumor prognostic marker results in poor specificity and a low positive rate in clinical application [15]. In our study, we demonstrated that expression of Topo IIα protein, as well as serum CEA and CA199, differentiation, and Dukes' stage were independent predictors of prognosis in colorectal cancer (Table 3). So the combination of the above five factors may be more valuable in prognosis prediction and treatment selection for colorectal cancer patients.

Tumor stage-related factors are very important for prognosis prediction and treatment selection in colorectal cancer. The accuracy rates for evaluation of invasion depth (T stage) and lymph node status (N stage) were reported to be 53–94% and 56–72% for preoperative CT, 55–89% and 60–83% for preoperative MRI, and 63–93% and 61–80% for preoperative endorectal ultrasound [16]. In a word, all of the preoperative images are not accurate enough in assessment of tumor stage. For early colorectal cancer detected by colonoscopy, it is difficult to get accurate information concerning tumor stage as T stage and N stage preoperatively due to relatively inaccurate preoperative imaging. Consequently, it is difficult to choose between microinvasive endoscopic resection and traditional macroinvasive resection for early colorectal cancer. Endoscopic resection may be attractive because of sooner recovery and minimal perioperative complications, but it is also a potentially risky option because lymph nodes may be involved even in well-differentiated small colorectal cancer [17]. Furthermore, if a colorectal polypus was resected under endoscopy and proved to be malignant lesion by biopsy-based pathology, it would be still impossible to get enough information concerning T stage and N stage from the resected specimens. In addition, there was no reliable prognostic biological marker, other than tumor differentiation, lymphatic or vascular invasion, and resection margin [5, 6]. So it is difficult to determine whether salvage laparotomy is necessary or not. A reliable prognostic biological factor is needed to aid in treatment selection for these two kinds of colorectal cancer patients. Our findings showed that Topo IIα expression was significantly related with T stage and N stage, which meant that expression of Topo IIα was predictive of tumor stage. So, expression of Topo IIα is useful for prognosis prediction and treatment selection in early colorectal cancers and malignant colorectal polyps resected under endoscopy, whose tumor stage-related factors are unavailable.

Overexpression of Topo IIα was proved to be related with higher sensitivity to Topo IIα-inhibiting drugs in testis cancer and bladder cancer cells in vitro [18]. In another word, Topo IIα may be an antioncogene rather than an oncogene, which is also supported by our study. The mechanism of overexpression of Topo IIα in colorectal cancer remains unknown. Expression of Topo IIα may be altered by activity at a DNA level or posttranslational modifications [13]. Kim et al. [19] found that mRNA expression of Topo IIα gene in colon cancer tumor tissue was higher than that in normal tissues. Further investigation of the mechanism of Topo IIα in the development and progression of human colorectal cancer is warranted.

In summary, our investigation demonstrates that expression of Topo IIα is a valuable prognostic indicator for colorectal cancer. And expression of Topo IIα is predictive of tumor stage, so it is especially useful in prognosis prediction and treatment selection for early colorectal cancer and malignant colorectal polypus resected under endoscopy, whose tumor stage-related factors are unavailable. Knowing that expression of Topo IIα protein, as well as serum CEA and CA199, differentiation, and Dukes' stage are independent prognostic factors for colorectal cancer, combination of these five factors may be more valuable in prognosis prediction and treatment selection in colorectal cancer. Further investigation of the mechanism of Topo IIα in human colorectal cancer is warranted.

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Conflicts of interest statement No conflicts of interest exist in the submission of this manuscript.

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