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Original Article

Macroscopic serosal classification of colorectal cancer and its clinical significance

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Abstract: Background: Macroscopic serosal classification of gastric cancer has been reported in previous studies, but rarely reported about it of colorectal cancer. The purpose of this study was to propose a macroscopic serosal classification of colorectal cancer and to investigate clinical significance of this classification. Materials and methods: Morphologic features of colorectal cancer were analyzed according to the macroscopic serosal appearance and clinicopathologic characteristics of these patients were retrospectively reviewed. Microscopic serosal structure was compared between different types under light microscope and transmission electron microscope. Results: Macroscopic serosal classification was divided into normal type, reactive type, nodular type and colloid type according to the macroscopic serosal appearance and microscopic structure. There were significant differences in tumor size, tumor gross type, histological type, histological grade, tumor necrosis, pT stage, number of nodes metastasis, lymph node metastasis ratio, pN stage, M stage and peritoneal metastasis between patients with different serosal types. Univariate analysis of prognosis revealed macroscopic serosal classification as one of factors significantly correlated with patient survival. However, multivariate analysis only revealed TNM stage significantly correlated with patient survival, while macroscopic serosal classification did not, maybe due to insufficient samples. Conclusions: Macroscopic serosal classification of colorectal cancer is preliminarily defined and divided into four types. Different macroscopic serosal types indicate different clinicopathologic features and correlate with prognosis of patients with colorectal cancer, but still cannot be proven as an independent factor.

Keywords: Colorectal cancer, macroscopic serosal classification, clinicopathologic characteristics, prognosis

Introduction

Macroscopic serosal classification (MSC) of gastric cancer was defined firstly in 1986 by professors of our department, based on the observation of more than 200 patients before 1986 and had reached agreement among all the surgeons and pathologists in First Affiliated Hospital of China Medical University in that year. But this study was published in Chinese journal and had not be known worldwide [1]. MSC of gastric cancer was classified as normal type, reactive type, nodular type, tendonoid type and color-diffused type, playing a critical role in predicting peritoneal micrometastasis. Compared with other methods, MSC was more sensitive than peritoneal cytological examination of peritoneal wash samples and more convenient than quantitative detection of the expression level of CEA mRNA which need a complex and time-consuming procedure. Further analysis also revealed that MSC was an independent predictor for both peritoneal recurrence and prognosis [2]. Besides peritoneal metastasis, lymph node metastasis also had been confirmed to have some relationships with serosa invading and MSC of gastric cancer [3, 4].

Colorectum as a distal part of digestive system has some similarities in the structure and biological behavior with stomach. But very few studies focus on analyzing clinicopathologic features and prognosis according to serosal invasion of colorectal cancer, more rare for classifying macroscopic serosal type. It may because that approximately half side of colorectum was wrapped by mesentery or in retroperitoneum, so near a half of colorectal cancer

couldn't be viewed in the side of serosa. However, for those patients that lesions invade serosal side, macroscopic serosal appearance will be observed firstly by surgeons when they perform the operation. If macroscopic serosal type correlates with clinicopathologic characteristics and can predict the prognosis of patients with colorectal cancer, it can determine the extent of rational resection and application of other anti-cancer treatments during surgery. In the present study, we propose a macroscopic serosal classification (MSC) of colorectal cancer according to morphology and structure of serosa, then hypothesize different macroscopic serosal types have different clinicopathologic characteristics and prognosis. Based on light microscope (LM) and transmission electron microscope (TEM), we compared microscopic serosal structure and components between different macroscopic serosal types. Furthermore, we investigated clinicopathologic characteristics and prognosis through a retrospective analysis of 213 patients with colorectal cancer.

Materials and methods

Patients with colorectal cancer underwent surgery at department of colorectal surgery, Liaoning Cancer Hospital & Institute during December 2010 to September 2011 were entered into this study. A total of 213 cases was suitable to analyze, 129 were men and 84 were women, with an age range from 28 to 86 years old (median 60). The change of bowel habits and bloody stool were the original symptom. Preoperative colonoscopy was taken to confirm tumor site and pathological diagnosis by biopsy. Application of computed tomography, ultrasound and magnetic resonance or other imaging techniques was to explore invasive depth of tumors, lymph node and distant metastasis. Histological staging was in accordance with the latest 7^{th} edition of the UICC TNM system (T is primary tumor; N is regional lymph nodes; M is distant metastasis), with a total of four stages [5]. I to IV stage were 26 cases (12.2%), 69 cases (32.4%), 83 cases (39.0%) and 35 cases (16.4%), respectively. Among patients of IV stage, there were 13 cases of peritoneal metastasis, accounting for 6.1% of the total. All patients were firstly treated with curative resection for primary tumor, followed with appropriate chemotherapy or radiation regimen according to TNM stage [6]. Resections were deemed curative when no gross residual disease was evident at the time of operation, with tumor-free margins on histological examination [7].

Patient selection criteria: (1) primary colorectal cancer, with or without metastasis; (2) lesions were located on the serosal side; (3) curative resection for primary tumor, regardless of metastasis; (4) without preoperative therapy; (5) patients undergoing elective surgery. The following criteria were excluded: (1) tumor directly invaded or was adherent to other organs or structures (T4b); (2) palliative, debulking surgery for primary tumor; (3) with other malignant tumors synchronously; (4) history of abdominal operation.

The type of serosa was prospectively determined according to their macroscopic appearance by three surgeons, who had experiences in more than fifty surgeries of colorectal cancer, immediately after the abdominal cavity being opened. If there was co-existence serosa type in one lesion, only the type that occupied the maximal area was recorded for analyzing. The same time we obtained a piece of serosa with 1.0-cm diameter and 0.2-cm thickness and divided it into three parts after rinsed in physiological 0.9% NaCl solution. One part fixed in 2.5% glutaraldehyde buffered with 0.1 M phosphate (pH 7.4) for transmission electron microscopy and the second part fixed in 10% formalin for light microscopy. The third one was stored in -80°C ultra-low temperature freezer (MDF-382E, SANYO, Japan), in order to extract proteins and mRNAs from serosal tissues for further study in the future.

Microscopic serosal appearance of 10% formalin-fixed tissues were observed with LM by experienced pathologist using hematoxylin-eosin staining as previously described [8]. Longitudinal sections of lesions after fixation, staining and a series of steps, were also observed with LM by the same pathologist. TEM sample preparation: the specimens fixed in 2.5% glutaraldehyde buffered, after fixation for 2-3 hours in 1% OsO_4, were rinsed with 0.1 M phosphate and dehydrated three times in an ascending ethanol series (50, 70, 90, and 100%), then embedded by Epon 812. The semithin sections (about 50 nm thickness) obtained by the slicer (PowerTome-XL, RMC company, US), double stained with 3% lead citrate and uranyl acetate, were visualized with TEM (H-

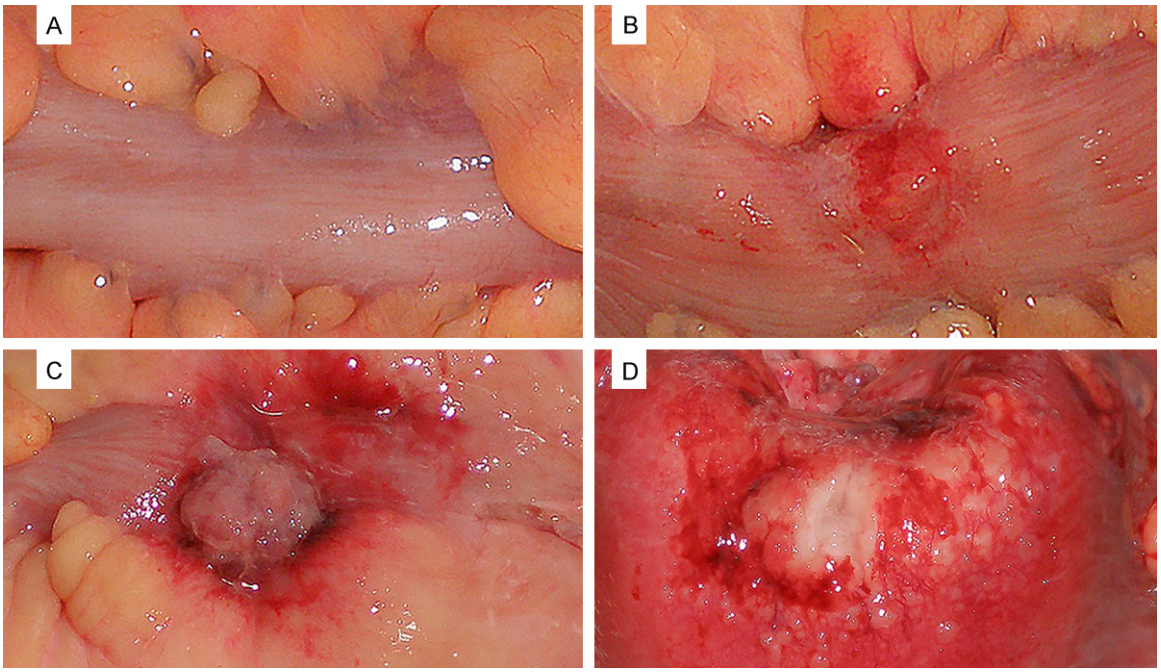

Figure 1. Macroscopic view of serosal classification. A. Normal type: normal serosal surface; B. Reactive type: red serosal changes, with obscure boundary; C. Nodular type: obvious tumor nodules protruding the surface of serosa; D. Colloid type: colloid changes predominantly, accompanied with massive small peritumoral nodules.

7650, Hitachi, Japan) in the accelerating voltage at 80 kV.

Clinicopathologic features such as sex, age, tumor size, tumor site, tumor gross type, histological type, histological grade, tumor necrosis, pT stage, number of nodes retrieved, number of nodes metastasis, lymph node metastasis ratio, pN stage, M stage and peritoneal metastasis were compared between patients with different serosal appearance. Univariate and multivariate analysis were applied for all 213 patients that were followed up completely, to identify the significant factors correlated with survival.

Follow-up of survival for the entire study population was obtained in household registration system at the Public Security Bureau, so the follow-up rate was 100%. Overall survival was defined as the time from surgery to the last follow-up (October 18, 2014) or patient death. Mean and median follow-up periods were 36.7 months and 40.7 months (range: 0.5-51.1 months), respectively.

This study was approved by the Research Ethics Committee of Liaoning Cancer Hospital & Institute (Shenyang, China). Written informed consent was obtained from all patients.

Statistical analysis

All the statistical analyses and graphics were performed with the SPSS for windows 19.0 statistical package (SPSS Inc., Chicago, IL). The mean of continuous data was expressed as $\bar{x} \pm s$ and categorical data was expressed as rate or percentage. In univariate analysis, two-tailed chi-square test for categorical variables and two-tailed t-test for continuous variables (Kruskal-Wallis test for heterogeneity of variance) were used for statistical comparisons. Overall survival rates were determined using the Kaplan-Meier estimator, an event being defined as death from a cancer-related cause. The log-rank test was used to identify differences between the survival curves of different patients groups. Multivariate Cox regression was used to identify independent factors correlated with prognosis. For all analyses, only P values < 0.05 were considered significant.

Results

Determination of macroscopic serosal classification

Referring to the articles about macroscopic serosal classification of gastric cancer written by Chen [1] and Sun [2], and summarizing the

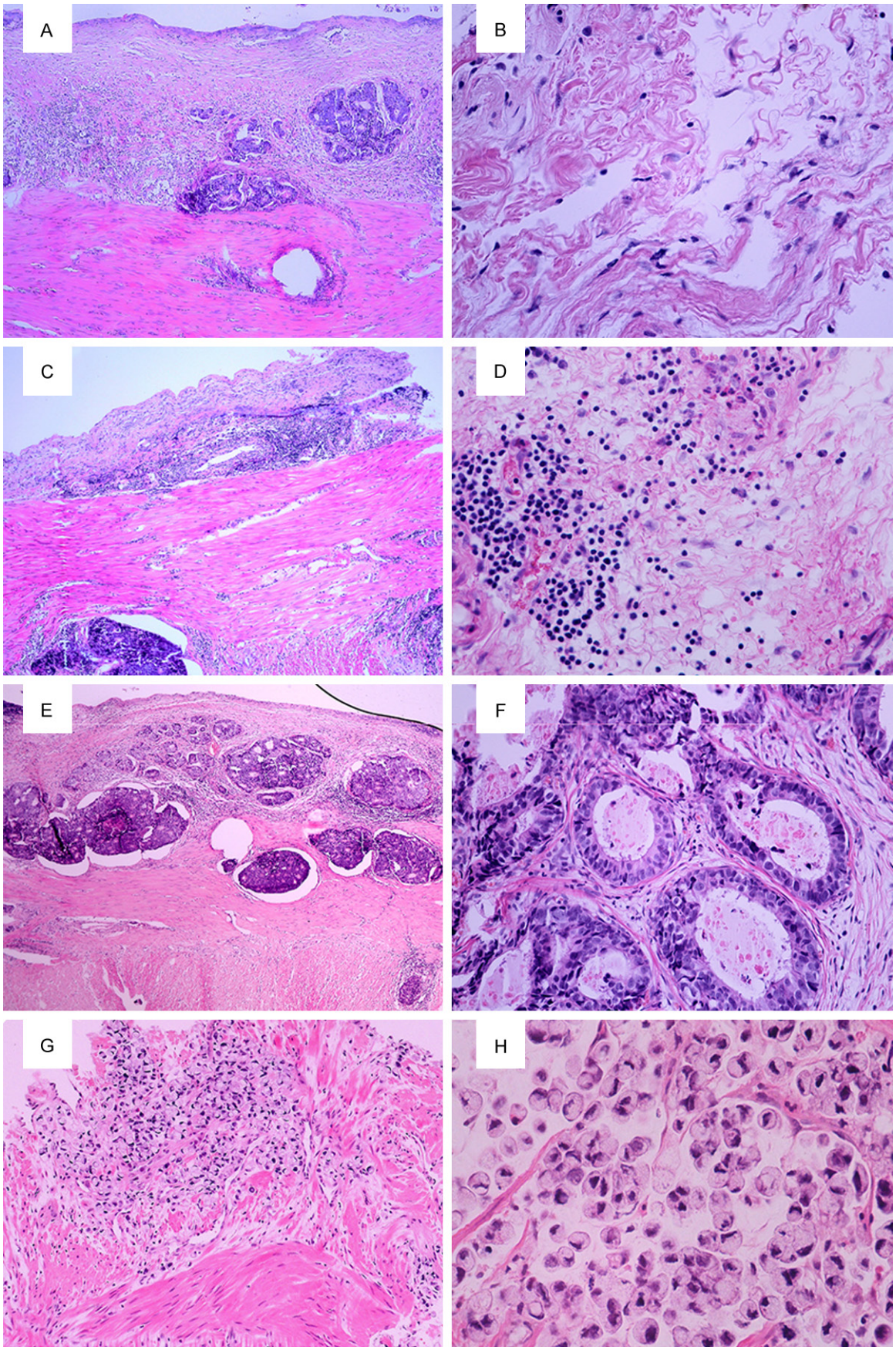
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Figure 2. Microscopic serosal appearances and structure observed with LM (representative sections, not for all). A. Normal type viewed in longitudinal section, tumor nests invaded into serosa but the surface of serosa was unchanged (H.E. ×100); B. Normal type viewed in cross section of serosa, the main structure of serosa included mesothelial cells and fibroblasts, accompanied with numerous collagenous fibers (H.E. ×400); C. Reactive type viewed in longitudinal section, a tumor nest invaded muscularis propria and a large number of inflammatory cells infiltrated subserosa (H.E. ×100); D. Reactive type viewed in cross section of serosa, a large number of inflammatory cells (lymphocytes) were among the collagenous fibers (H.E. ×400); E. Nodular type viewed in longitudinal section, approximately a dozen nests infiltrated serosa but not penetrated (H.E. ×100); F. Nodular type viewed in cross section of serosa, the serosal surface was covered with glandular tumor nests (H.E. ×400); G. Colloid type viewed in longitudinal section, the structure of serosa outside of muscularis propria was not clear and full of signet ring cells (H.E. ×200); H. Colloid type viewed in cross section of serosa, the serosal surface was covered with signet ring cells (H.E. ×400).

characteristics of hundreds cases of colorectal cancer serosal change, we preliminary divided macroscopic serosal appearance into four types: normal type, reactive type, nodular type and colloid type. Normal type: the serosal surface, which color and shape were completely consistent with normal serosal tissues, was smooth, soft touch and pressed without depression (**Figure 1A**). Reactive type: the serosal surface was red or white changes, with obscure boundary, touched smoothly or slightly rough (**Figure 1B**). Nodular type: there were some protruding nodules scattered or accumulated together on the surface of serosa, with hard and rough texture (**Figure 1C**). There were also some serosa sunken changes, due to serosa contracture. Colloid type: obvious colloid change was viewed on the serosal surface (area > 50% lesion), with or without tumor nodules or necrosis (**Figure 1D**).

Observation under LM and TEM

The microscopic serosal structure and characteristic components of different serosal types were observed using LM and TEM. What we found with LM was as follows. Normal type: observed in longitudinal sections, the morphology and structure of the serosal surface were no change and tumor cells didn't penetrate the serosa. The outermost layer of serosa was monolayer mesothelial cells, between it and muscularis propria was full of collagen fibers. In cross sections, only mesothelial cells, fibroblasts and collagen fibers could be observed. Reactive type: there were plenty of inflammatory cells (mainly lymphocytes) accumulated among collagen fibers in serosa and tumor cells didn't penetrate the serosa or penetration was invisible. Nodular type: the whole serosal layer was permeated with glandular cancer nests, with or without tumor penetration of serosal surface. Colloid type: normal serosal structure outside of muscularis propria disappeared, replaced by mucin pool in extracellular space, or irregular close signet ring cells, or some necrosis tissues and cell fragments. **Figure 2** shows some representative sections observed with LM.

Characteristics of different serosal types observed with TEM are detailed described in **Figure 3**. Consistent with the results observed with LM, the characteristic cells of normal type were mesothelial cells, with the diameter of 5~10 μm of oval shape. On cell surface, numerous microvilli were noted. In reactive type, the dense small lymphocytes (diameter < 5 μm) between mesothelial cells and collagen fibers played the crucial structural role differing from other three types. Huge special-shaped tumor cells consisted of the main structure of nodular type. And in colloid type, large amount of intracellular or extracellular mucinous granules were characteristic components which widely existed in signet ring cell cancer or mucinous adenocarcinoma.

Clinicopathologic characteristics

Clinicopathologic characteristics of patients with different serosal types are listed in **Table 1**. As shown, there were significant differences in tumor size, tumor gross type, histological type, histological grade, tumor necrosis, depth of invasion (T stage), number of nodes metastasis, lymph node metastasis ratio, pN stage, M stage and peritoneal metastasis between patients with different types of serosa.

Concerning the tumor site between four types of MSC, there were no significant differences. But comparison of tumor site between non-colloid type and colloid type, we could revealed that colorectal cancer with colloid type was

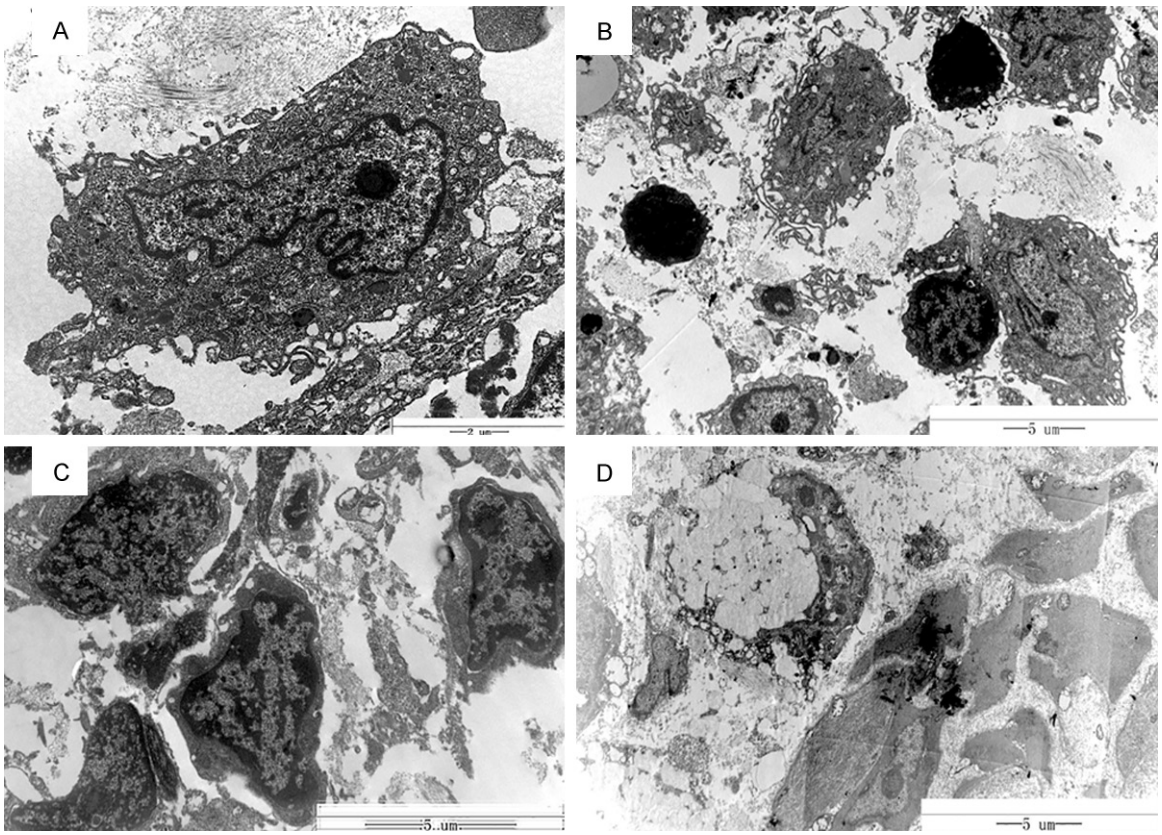

Figure 3. Microscopic serosal structure and characteristic cells observed with TEM. A. Normal type: typical mesothelial cell was observed in serosa, with numerous microvilli in its surface. Nucleus was in center with a prominent nucleolus and stained nuclear membrane. Abundant cytoplasm contained a wealth of endoplasmic reticulum and mitochondria. Collagen fibers existed outside of cell. B. Reactive type: lymphocytes were visible among mesothelial cells and fibroblasts in serosa, containing a large nucleus and less cytoplasm. Nucleus accounted for most of the cell, accompanied with heterochromatin that obviously accumulated in perinuclear. C. Nodular type: three intact tumor cells were visible, with large nucleus, less cytoplasm and stained chromatin. D. Colloid type: a signet ring cell penetrating muscularis propria reached serosa. It contained intracytoplasmic mucinous granule, pushing its nucleus to one side.

more likely to occur in the proximal intestine (P = 0.043) (**Table 2**).

Considering the morphology, structure, and clinicopathologic characteristics between MSC, it was more easily to summarize that the malignant degree of colorectal cancer with nodular and colloid types was higher than other two types.

So, we singled out these two types of cancers for further analysis. Comparing the depth of invasion and lymph node metastasis (LNM) between two types, no significant differences were noted (P > 0.05), but the *p* value of LNM was very close to significance (P = 0.051) (**Table 3**). The ratio of peritoneal metastasis between two types was different significantly (P = 0.003) (**Table 4**), and we just compared the tumors that penetrated the surface of serosa (pT4). Because from the previous studies, tumor cells penetrating the serosa had the probability to cause peritoneal metastasis and seldom exfoliation cancer cells passed through other access such as the surface of lymph node [9].

Prognosis of patients

The 1- and 3-year survival rates of the entire patients followed up were 92.0% and 77.3%, respectively. The overall average survival time was estimated to be 43.3 months, while the median survival time cannot be estimated. Univariate analysis of prognostic factors identified tumor gross type, histological type, histological grade, pT stage, pN stage, M stage, TNM

Table 1. Comparison of clinicopathologic features between patients with different types of serosa

	Normal type	Reactive type	Nodular type	Colloid type	Statistics	P
Sex						
Male	27	42	35	25	$\chi^2 = 3.068$	0.381
Female	15	20	30	19		
Age (yrs)	57.81±10.77	62.40±11.26	60.12±11.02	59.73±12.49	F = 1.350	0.259
Tumor size (cm)	3.64±1.41	4.65±1.66	5.05±1.69	6.02±2.72	F = 11.787	0.000
Tumor site						
Colon	19	21	31	26	$\chi^2 = 6.785$	0.079
Rectum	23	41	34	18		
Tumor gross type						
Protruded	26	8	15	3	$\chi^2 = 76.599$	0.000
Ulceration	16	54	30	30		
Infiltrative	0	0	20	11		
Histological type						
Non-MUC	36	55	53	9	$\chi^2 = 73.156$	0.000
MUC	6	7	12	35		
Histological grade						
Well	24	10	10	2	$\chi^2 = 71.556$	0.000
Moderately	16	52	49	27		
Poorly	2	0	6	15		
Tumor necrosis						
Yes	6	43	38	18	$\chi^2 = 33.999$	0.000
No	36	19	27	26		
UICC T stage						
T1/T2	26	5	0	0	$\chi^2 = 116.158$	0.000
T3	16	47	38	22		
T4	0	10	27	22		
Number of nodes retrieved	20.00±17.10	19.58±8.56	22.26±8.56	24.64±10.61	F = 2.127	0.098
Number of nodes metastasis	1.62±4.10	2.16±4.08	5.86±6.94	9.18±10.12	$\chi^2 = 42.420^*$	0.000
LNM ratio (%)	8.15±19.06	12.05±21.05	24.74±27.91	37.31±33.90	$\chi^2 = 38.163^*$	0.000
UICC N stage						
N0	32	35	23	8	$\chi^2 = 47.222$	0.000
N1	6	18	11	12		
N2	4	9	31	24		
UICC M stage						
M0	42	53	52	31	$\chi^2 = 14.537$	0.002
M1	0	9	13	13		
Peritoneal metastasis						
Yes	0	0	3	10	$\chi^2 = 28.229$	0.000
No	42	62	62	34		

*Kruskal-Wallis test of mean ± SD. MUC: Mucinous adenocarcinoma; LNM: Lymph node metastasis.

stage and MSC were significantly correlated with survival (**Table 5**). Putting all above significant variables selected by univariate analysis into Cox regression model, and after interaction of T, N and M stage with TNM stage, we drew a conclusion by multivariate analysis that TNM stage was the unique independent prognostic factor (**Table 6**).

Comparing survival rates according to MSC, survival curves indicated that colloid type was the worst, followed by nodular type, reactive

Table 2. Comparison of tumor site between non-colloid type and colloid type

Tumor Site	Serosal type		OR (95% CI)	χ^2	*P* value
	Non-Colloid Type	Colloid Type			
Colon	71	26	0.502 (0.256-0.984)	4.106	0.043
Rectum	98	18			

Table 3. Comparison of UICC T stage and LNM between nodular type and colloid type

	Serosal type		OR (95% CI)	χ^2	*P* value
	Nodular Type	Colloid Type			
UICC T stage					
T3	38	22	1.407 (0.652-3.039)	0.759	0.384
T4	27	22			
LNM					
No	23	8	2.464 (0.983-6.180)	0.384	0.051
Yes	42	36			

LNM: Lymph node metastasis.

Table 4. Tumor penetrating serosal surface (T4) and peritoneal metastasis

	Normal type	Reactive type	Nodular type	Colloid type	χ^2	P
Number of T4	0	10	27	22	—	—
Number of PM	0	0	3	10	—	—
Ratio of PM (%)	0	0	11.11	45.45	11.726	0.003

PM: Peritoneal metastasis.

type and normal type (**Figure 4**). Survival rate of different TNM stage was also compared by log-rank test described by **Figure 5**.

Discussion

Typically, macroscopic classification of colorectal cancer has been divided into three types according to its gross appearance, including protruded, ulceration and infiltrative, which was concluded from the morphologic observation of mucosal surface. This macroscopic classification has been indicated to have some certain correlation with the patient prognosis that prognosis of patients with ulceration type is in an intermediate position between the best of protruded type and the worst of infiltrative type [10, 11]. Morphological studies seem to be superficial, but in fact it is closely related to the biological behavior, differentiation, growth pattern, and many other features of tumor cells [12]. For surgeons, the first sight when entering abdominal cavity at the beginning of surgery is serosal appearance instead of mucosa, then to conduct the appropriate surgery and other clinical decision making partly according to serosal changes, especially more significant to those patients without distant metastasis. So, serosal morphological characteristics of colorectal cancer are particularly important. The present study, which is firstly reported worldwide, merely focused on the macroscopic serosal classification (MSC) and investigated clinicopathologic characteristics and prognosis of different MSC.

Through summarizing macroscopic serosal appearances of colorectal cancer and observing microscopic characteristics with LM and TEM, MSC was preliminarily classified as normal type, reactive type, nodular type and colloid type. From the results, there was no statistical significance in tumor site between patients with different types. But compared colloid type with other three types, it was more frequently found in the proximal colon, mainly due to the serosal histological components of colloid type which composed by mucinous adenocarcinoma, signet ring cell cancer and a small part of necrosis. Previous studies comparing clinicopathologic characteristics of mucinous carcinomas (MCs) with non-mucinous carcinomas (NMCs) of the colon and rectum, indicated that MCs occurred in the right colon significantly more frequently than did non-mucinous carcinomas (NMCs) [13, 14]. Similar study by Hugen et al. reported that patients with signet ring cell cancer presented more frequently in the proximal colon (57.7 vs. 32.0%) than patients with adenocarcinoma cancer [15]. However, tumor location of signet ring cell cancer remains controversial. Song et al. revealed signet ring cell tumors were more

Table 5. Univariate analysis of prognostic factors for 213 patients

Factors	Number	Survival rate (%)		χ^2	P
		1-year	3-year		
Sex					
Male	129	94.6	80.6	1.741	0.187
Female	84	88.1	72.6		
Age (year)					
≤ 60	113	93.8	77.9	0.143	0.705
> 60	100	90.0	76.9		
Tumor size (cm)					
≤ 5	146	93.2	76.7	0.143	0.705
> 5	67	89.4	75.5		
Tumor site					
Colon	97	86.6	73.2	2.357	0.125
Rectum	116	96.6	81.0		
Tumor gross type					
Protruded	52	98.1	96.2	28.500	0.000
Ulceration	129	93.0	77.3		
Infiltrative	32	78.1	46.1		
Histological type					
Non-MUC	160	94.4	80.6	4.772	0.029
MUC	53	84.9	67.8		
Histological grade					
Well	46	95.7	91.3	7.025	0.030
Moderately	144	93.1	75.7		
Poorly	23	78.3	60.3		
Tumor necrosis					
Yes	78	90.4	78.5	0.393	0.531
No	135	94.9	75.6		
UICC T stage					
T1/T2	31	96.8	93.5	7.208	0.027
T3	123	91.9	78.0		
T4	59	89.8	67.6		
UICC N stage					
N0	98	96.9	92.9	32.090	0.000
N1	47	95.7	76.6		
N2	68	82.4	55.5		
UICC M stage					
M0	178	94.4	84.3	42.273	0.000
M1	35	80.0	42.5		
TNM stage					
I	26	96.2	96.2	49.463	0.000
II	69	97.1	91.3		
III	83	91.6	74.7		
IV	35	80.0	42.5		
MSC					
Normal type	42	95.2	88.1	10.968	0.012
Reactive type	62	95.2	82.3		
Nodular type	65	92.2	76.6		
Colloid type	44	86.4	65.1		

MSC: Macroscopic serosal classification.

commonly found in the rectum than mucinous and non-mucinous adenocarcinoma [16].

Regarding clinicopathologic characteristics of MSC, it is very easy to discriminate that nodular type and colloid type are more malignant than normal type and reactive type, according to the aspects in the depth of invasion, lymph node and distant metastasis. Nodular type and colloid type invade deeper than other two types, but without significant difference within themselves. Lymph node metastasis compared between nodular type and colloid type, there is a worse trend emerged for colloid type, but it has not result in a statistically significant difference. Previous studies revealed that mucinous and signet ring cell tumors presented in a later stage more often than non-mucinous adenocarcinoma, including lymph node involvement [14, 16]. Maybe the differences of LNM between nodular type and colloid type will be statistical significance with samples increasing in the current study. Distant metastasis between nodular type and colloid type has no significant differences, excluding the peritoneal metastasis which has a significant higher proportion in colloid type than that in nodular type. Free cancer cells penetrating serosa may be the crucial step and main reason for the development of peritoneal metastasis. In accordance with it by histological examination, the depth of invasion should equal or exceed pT4 besides changes in macroscopic serosal morphology. So we further compared the patients with pT4 of different serosal types, peritoneal metastasis rate of colloid type was still significantly higher than that of other types. In other words, free cancer cells of colloid type have stronger ability to exfoliate from the lesions where tumors penetrate the serosa and to result in metastatic carcinogenesis in peritoneal cavity, than those of nodular type and reactive type. Analyzing from the view of histological components, colloid type is composed by mucinous carcinoma and signet ring cell cancer, which more frequently had

Table 6. Multivariate analysis of prognostic factors for 213 patients

Variable	B	SE	Wald	df	Sig.	Exp (B)	95.0% CI for Exp (B)	
							Lower	Upper
Tumor gross type	0.357	0.271	1.739	1	0.187	1.429	0.841	2.429
Histological type	0.510	0.294	3.015	1	0.082	1.665	0.936	2.960
Histological grade	0.168	0.289	0.338	1	0.561	1.183	0.671	2.086
TNM*T interaction	-0.097	0.086	1.281	1	0.258	0.907	0.767	1.074
TNM*N interaction	0.135	0.074	3.350	1	0.067	1.144	0.990	1.322
TNM*M interaction	0.086	0.142	0.370	1	0.543	1.090	0.826	1.439
TNM stage	0.689	0.275	6.289	1	0.012	1.991	1.162	3.411
MSC	-0.050	0.203	0.061	1	0.805	0.951	0.639	1.416

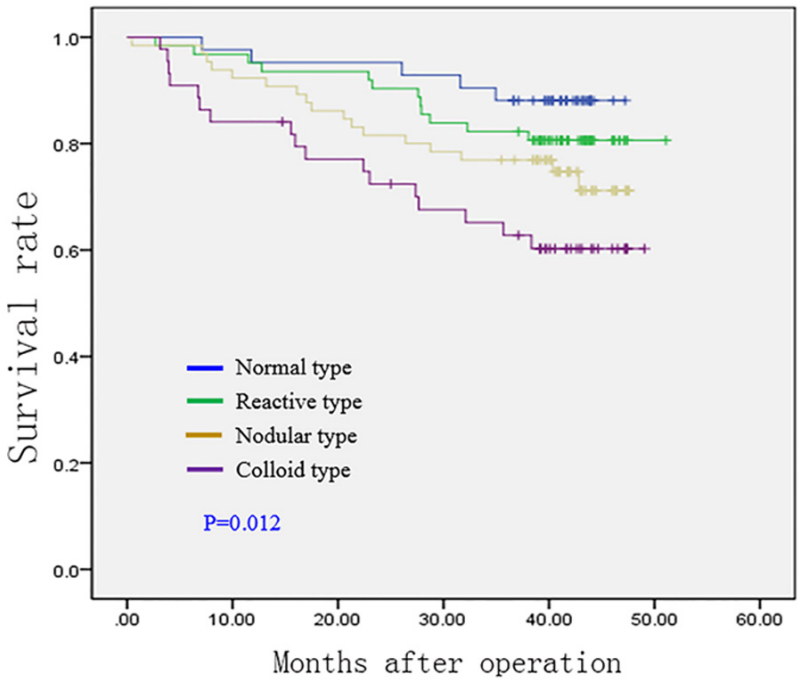

Figure 4. Survival curves of patients after operation according to macroscopic serosal classification (n = 213, P = 0.012).

peritoneal metastases compared with classic adenocarcinoma cancer [17]. Therefore, one of meaningful contents about application of MSC is to make intraoperative decision regarding proper treatment strategy. For patients with colloid type of colorectal cancer, besides conventional surgery including lymph nodes dissection, it is necessary to use intraperitoneal chemotherapy or other methods for preventing peritoneal metastasis.

Our further analysis confirmed that MSC, one of predictors for prognosis of patients with colorectal cancer, will become a positive complement of traditional prognostic factors. Clinicopathologic characteristics and histological components of different serosal types decide different prognosis, such as signet ring cell and mucinous components in colloid type differ from classical adenocarcinomas in nodular type [18, 19]. But in the present study, MSC is still not an independent predictor according to statistical results, maybe due to the insufficient sample. While TNM stage, the unique independent prognostic factor for patients with colorectal cancer, is always in an irreplaceable position for prognostic evaluation.

Of course, MSC has some disadvantages. For example, a small part of patients with pT3 or pT4 can't be accurate judgment quickly by surgeons when entering abdominal cavity through serosal changes in both nodular type and colloid type, even in a few reactive type. Then the method of serosal cytologic smears could be applied to confirm the depth of invasion as an effective supplement of MSC [20]. Serosal cytologic smears could estimate whether tumor cells easily exfoliate from the serosal surface into peritoneal cavity. Panarelli et al. concluded that cytologic smears improve detection of peritoneal penetration among pT3 tumors compared with histology alone. Tumors close (≤ 1 mm) to a fibroinflammatory tissue reaction on the serosa are likely associated with peritoneal involvement by cancer. Peritumoral abscesses that communicate with the serosa and hemorrhage or fibrin on the serosa also predict cancer involvement of the peritoneum [21]. According to macroscopic appearance of abscesses, which is often associated

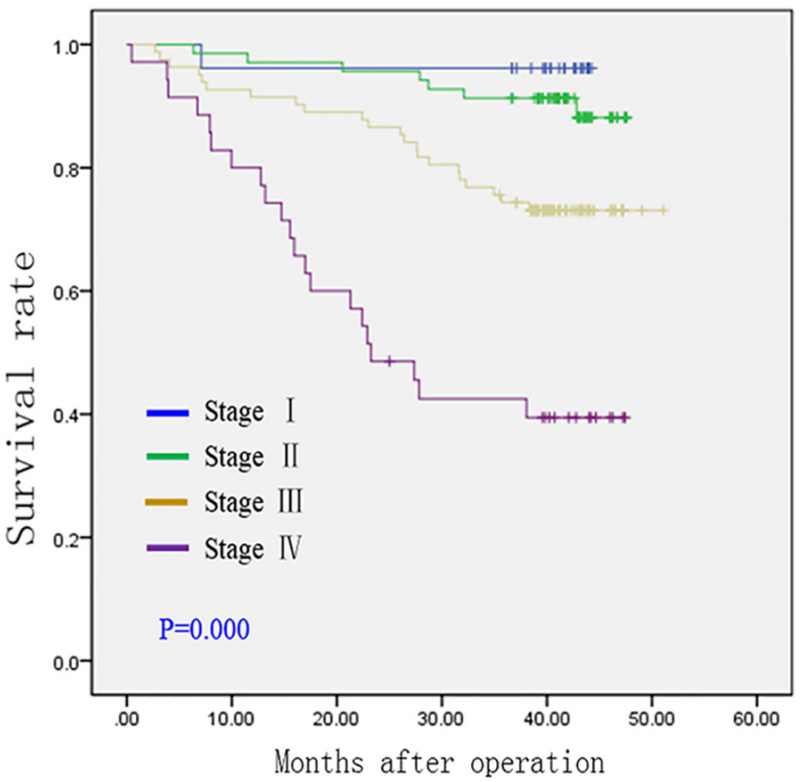

Figure 5. Survival curves of patients after operation according to TNM stage (n=213, P=0.000).

with necrosis, we put it into colloid type. But without being classified about serosal hemorrhage and fibrin, further studies need to be carried out to make MSC more refined and detailed.

The present study provided the morphological difference of serosal appearance from macroscopic observation to light microscopy, then indicated the components and structural differences from light microscopy to transmission electron microscopy, eventually summarized the clinical significance of MSC through retrospective analysis of clinicopathologic materials. Looking forward for further study, in addition to making definition and classification of MSC detailed, we would analyze the molecular changes and compositions in different serosal type from histological level, in order to provide partial supports for molecular serosal classification of colorectal cancer.

In conclusion, we found MSC of colorectal cancer, which was preliminarily defined and classified as normal type, reactive type, nodular type and colloid type. Patients with different serosal types have different clinicopathologic features and prognosis. The information about serosal type and invasion, which can be known at the first time by surgeons in the beginning of the surgery, aids them in making proper intraoperative decisions including surgical performance and other anti-cancer treatments.

Disclosure of conflict of interest

None.

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