Prognostic Significance of Microvessel Density and Mast Cell Density for the Survival of Thai Patients with Primary Colorectal Cancer

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Background: *Angiogenesis has been found to be a reliable prognostic indicator for several types of malignancies. In colorectal cancer, however, there has been controversy as to whether there is a correlation between this feature and the tumors' behavior.*
Objective: *Determine the correlation between microvessel density (MVD) and mast cell density (MCD) in order to evaluate these factors in terms of their prognostic relevance for primary colorectal carcinoma in Thai patients.*
Material and Method: *One hundred and thirty colorectal carcinoma patients diagnosed between January 2002 and December 2004 were identified. Eleven patients were excluded from the present study due to recurrence of colorectal carcinoma in eight cases whereas pathologic blocks were not found in three cases. One hundred and nineteen patients met all inclusion criteria and were included in the present study. Representative paraffin sections obtained by the tissue micro-array technique (9 x 5 arrays per slide) from areas of highest vascular density (hot spots) were prepared. Sections were immuno-stained by monoclonal anti CD 31 for microvessel and antibody mast cell tryptase for mast cell detections, respectively. Three readings at different periods of time under a microscopic examination of high power magnification were examined by a pathologist who was blinded to clinical data. The highest microvessel and mast cell counts were recorded as MVD and MCD. Patients were then divided into groups of high and low MVD and high and low MCD by median values (20.5 and 14.5, respectively). Overall survival of the patients in each group was estimated by the Kaplan-Meier Method while a multivariate Cox regression backward stepwise analysis was employed to find out independent prognostic factors.*
Results: *Significant positive correlation was found to exist between MVD and MCD in the hot spots ($R = 0.697$, $p < 0.0001$). Regarding their prognostic role, patients with tumors of low MVD (hypovascular) and low MCD (low mast cell counts) had significantly longer survival rates than those with hypervascular and high mast cell counts ($p < 0.0001$). The Multivariate Cox hazard showed that MVD and distance metastasis of cancer were independent poor prognostic factors to survival ($p = 0.036$ and $p = 0.024$, respectively).The patients with high MVD (hypervascular) tumors and with presence of distant metastasis had 1.9 and 2.5 times higher death rates than the corresponding hypovascular and non-metastatic groups, respectively during the period from January 2002 to September 2007.*
Conclusion: *Assessment of microvessel density in the invasive front of primary colorectal carcinoma could serve as useful prognosis tool of primary colorectal carcinoma in Thai patients.*

Keywords: *Angiogenesis, Anti CD 31, Antibody mast cell tryptase Colorectal cancer, Survival*

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Colorectal carcinoma is one of the most common neoplasms encountered in our modern world, affecting individuals in most developed countries and ranking second only to lung cancer as the cause of cancer-related mortality[1]. Although the prevalence of colorectal cancer in Thailand is generally low compared to other countries, current trends indicate that the numbers of colorectal cases are increasing rapidly in Bangkok. This is most likely due to high calories and lower fiber diets of people in urban areas. While Bangkok has the highest incidence rate for both sexes, colorectal cancer follows liver and lung cancer to rank third in frequency in males. The age-standardized incidence rate was 12.4 for males and 9.6 for females[2].

The biological behavior of colorectal carcinoma is related to clinical and pathological parameters wherein several parameters have been found to have prognostic value e.g. tumor stage[3], grade[4], presence of vascular invasion[5], tumor size and site and presence of perforation[6] wherein the most significant prognostic factor is tumor stage.

The development of liver metastasis has developed around 40% of colorectal carcinoma, which is the cause of death in the majority[7]. Lymph node involvement, however, does not always predict the risk of disease progression because it has been found that ten to thirty percent of colonic tumors will recur despite the absence of lymph node metastasis at the time of resection[8]. Angiogenesis as an indicator of the aggressiveness of the tumor and has been found to be an independent predictor of survival in cancers of the breast[9,10], prostate[11], lungs[12], skin[13], larynx[14], oral cavity[15], and bladder[16]. For colorectal carcinoma, however, the results have been inconsistent. Although several studies found correlations between high microvessel counts and more aggressive behavior[17-21], other studies have found no relationship between tumor angiogenesis and prognosis in colorectal carcinoma[22,23].

The role of mast cells in tumor behavior has remained speculative wherein several studies[24-26] have suggested that mast cells are implicated in mediating angiogenesis. Grutzkare et al reported that human mast cells expressed all known isoforms of the most potent angiogenesis factor (Vascular Endothelial Growth Factor)[27]. Moreover, Acikalin et al have demonstrated a clear correlation between higher numbers of mast cells and microvessels and their relation to poor prognosis in colorectal cancer[28]. Therefore, the association between mast cells, vasculature and increase rate of mast cell production during tumor growth, accompanied by neovascularization add support to this presumption[25,27].

The present article was aimed at investigating the microvessel and mast cell density in 119 cases of colorectal carcinoma patients by using immunohistochemistry methods to determine the correlation between the microvessel and mast cell density and to evaluate whether microvessel density (MVD) and mast cell densities (MCD) could predict the survival of Thai patients with colorectal carcinoma. Therefore, a look at the biological behaviors of colorectal carcinoma is essential to obtaining an understanding of the background and significance of this disease.

Material and Method

The present study obtained approval from the Ethics Committee for Research of Bangkok Metropolitan Administration. The authors originally identified 130 patients who had undergone tumor resections for carcinoma of the colon and rectum between January 2002 and December 2004 at Chareonkrung Pracharak Hospital, Bangkok Metropolitan Administration. None of these patients had received chemotherapy or radiotherapy before surgery. Eleven patients were excluded from the study due to recurrence of colorectal carcinoma in eight cases, whereas pathologic blocks were not found in three cases. One hundred and nineteen patients had complete access to clinicopathological data and were studied for MVD and MCD, however, the patients died during the periperative period and excluded for survival analysis.Total 116 patients had adequate follow-up information and were included in the present study.

Table 1 shows the distribution of the characteristics for the patients in the sample, which consisted of 68 men (57.1%) and 51 women (42.9%) ranging from 15 to 91 years in age (mean age: 60.5 $\pm$ 12.9; median age: 62 years). Seventy-two (60.5%) of the patients suffered from colon cancer and forty-seven (39.5%) suffered from rectal cancer. The patients were staged according to the criteria set forth by the American Joint Committee on Cancer[29]. The tumors were also graded in terms of well, moderate and poor differentiation by the WHO criteria. Many types of surgery were performed e.g. wedge-shaped excision, hemicolectomy, low anterior and anterior/posterior resection, etc. The patients had primary tumor resection with regional lymph node dissection, followed by chemotherapy (Fluorouracil combined with Leukovorin), depending on the pathological stage. In order to protect the rights of human subjects, informed consent was obtained from all patients prior

to participation in the present study. The progress of these patients was followed until 30 September 2007 wherein it was found that fifty-four patients (46.6%) survived during this period. The overall survival time of the postoperative patients ranged from 0.5 to 63.5 months.

Immunohistochemistry studies for CD31 antigen and mast cell tryptase

The tumor specimens that were obtained were fixed in 10% buffer formalin and embedded in paraffin. Full cross sections of each tumor for evaluation were used with hematoxylin-eosin staining. The sections were first screened at a low power field (x 40 magnification) to identify the areas of highest vascularization within the tumor's invasive front (hot spot). The tissue micro array technique was applied to diagnose tissues from the hot spot areas of 119 colorectal carcinomas in a technique that involved taking tissue cores from existing paraffin embedded by tissue extractors and injections of tissue cores into the holes of recipient paraffin blocks, each comprising of 45 holes (2 mm in diameter per hole) and arranged in 5 x 9 arrays.

The sections from the tissue in the micro array blocks of 119 colorectal carcinomas were studied for MVD and MCD by immunohistochemistry methods and the sections for examining MVD were stained by CD31 (Monoclonal Mouse Anti-human CD31 Clone JC/70 A) (Dako code No. M0823). The endothelial cells of the tumor vessels were highlighted by this method and the sections for the present study of mast cell density were stained by mast cell tryptase (Monoclonal Mouse Antibody Clone AA1) (Novocastra code NCL-MCTRYP). After deparaffinization in xylene and rehydration in ethanol, the antigen retrieved was treated with ethylene diamine tetraacetic acid (EDTA) in a pressurecooker at 125°C for 3 minutes under 20 psi pressure. The sections were then endogeneous peroxidase blocked for 10 minutes in 3% hydrogen peroxide in distilled water. Then the slides were processed according to standard immune histochemistry methods with the Envision (Dako code No.K4001). The monoclonal antibody, CD31 (JC/70A), and the monoclonal mouse antibody mast cell, tryptase, were diluted with Tris buffered saline solution at ratios of 1:200 and 1:700, respectively, after which, they were reacted with the tissue specimens for 60 minutes at room temperature and overnight at 4°C. Diaminobenzedine (DAB) was used as chromogen, followed by hematoxylin counterstaining. The sections were incubated with normal immune sera instead of the primary antibodies, to be used as a negative control.

Determination of microvessel density and mast cell density

The slides were scanned at low magnification (x 40 and x 100) to identify the areas of invasive carcinoma with the highest numbers of microvessels by a pathologist who had not been informed of the patient's data. Three readings at three different periods of the most vascularized area were obtained within a x 400 microscopic field i.e. x 40 objective lens and x 10 ocular lens corresponding to an investigated area of 0.192 mm^2. The method of microvessel counting was based on the study of Engel CJ et al[30], and the highest microvessel count was recorded as the microvessel density. Any brown-stained endothelial cell that had clearly separated from its adjacent microvessels was considered a single countable microvessel. Undefined endothelial cells that appeared to be fragments were not counted as microvessels. Branching structures were counted as a single vessel unless there was a break in continuity of the structure. The presence of a vessel lumen was not required to classify a structure as a vessel. All microvessel counts were made far from areas of inflammation, necrosis and ulceration due to increased microvessels independent of the tumor. MCD was evaluated the same technique of microvessel density.

Statistical analysis

Data was analyzed by parametric and non-parametric statistics, using SPSS 11.5 (Chicago, IL). Descriptive statistics were used for demographic data and summarized as mean with standard deviation (SD) or median with range for continuous variables and as number with percentage for discrete variables. The Kolmogorov-Smirnov, Lilliefors and Shapiro-Wilks' W-tests were used for analyzing the normality of the distribution. Then the parametric t-test and the Anova test were applied to the evaluation of significant differences among the mean ranks. Non-parametric Spearman's rank correlation was applied to study the correlation between MVD and MCD and cumulative survival curves were drawn by the Kaplan-Meier method. The difference between the curves analyzed by log-rank test employed an intercooled Stata version 9.2 (Stata Corp, College station, TX) and Cox's proportional hazard model of the StatView package was used for multivariate analysis performed with factors which were significant. Hazard ratio [HR] and their 95% confidence intervals were calculated and factor differences with $p < 0.05$ were considered statistically significant.

Results

In the present study, the samples were divided into hypovascular (< 20.5 vessels/HPF), and hypervascular (≥ 20.5 vessels/HPF) groups according to the median value of the microvessel counts (20.5). The cut-off value of 14.5 (the median value of the mast cell counts) divided the whole series into the tumors with low mast cell counts (< 14.5 cells/HPF) and high mast cell counts (≥ 14.5 cells/HPF). Although the sample retained for the study comprised 119 subjects, three more patients were excluded from the survival analysis due to perioperative death. The remaining 116 cases were followed until 30 September 2007.

The patient characteristics and clinicopathological details as shown in Table 1 indicate that 72 (60.5%) of the patients aged between 15-91 years old (mean: 60.5 ± 12.9; median: 62) suffered from colon cancer while 47 of these patients (39.5%) suffered from rectal cancer. The tumor locations were identified in numbers as follows: rectum (47 cases); sigmoid colon (34 cases); ascending colon (16 cases); cecum (7 cases); hepatic flexure colon (5 cases); transverse colon (5 cases); splenic flexure colon (4 cases); and descending colon (1 cases). The patients were predominantly classified as stage III in 57 cases (47.9%), moderate grade in 88 cases (73.9%); T3-T4 depth invasion in 104 cases (87.4%); lymphatic vascular invasion in 77 cases (64.7%); and perineural invasion in 48 cases (40.4%). The most prevalent symptom was obstruction, which was encountered in 59 cases (49.5%). Distant metastasis occurred in eight cases (6.7%) and, of those, seven had metastasized to the liver (87.5%).

The MVD detected by immunohistochemistry stains in the hot spots ranged from 5 to 44 vessels per x 400 (HPF) with a median of 20.50 vessels as shown in Fig. 1 where examples of high MVD can be seen. The tryptase positive mast cell density (MCD-tryp) in the hot spots ranged from 3 to 32 cells per HPF with a median of 14.5 as demonstrated in Fig. 2 where examples of high MCD can be seen. The correlation between MVD and MCD by Spearman's rank correlation test is displayed in Fig. 3 wherein a strongly significant correlation between MVD and MCD was obtained by $R = 0.697$ and $p < 0.0001$. The associations between MVD and MCD in the hot spots of the invasive front of the tumors with the clinicopathological factors are revealed in Table 2, wherein it can be seen that MVD and MCD in the most vascularized areas were statistically significantly associated with TNM staging between stages (except at stage I and stage II) which was also the case of all histological grades, depths of invasion (except T1 and T2), presence of lymphatic vascular invasion, presence of perineural invasion and distant metastasis. No statistically significant associations were observed between MVD and MCD in age groups, genders, or location between the colon and rectum. In terms of symptoms, the MVD in hot spots was statistically significant between the symptom of obstruction and other symptoms. However, no statistically significant differences of MCD between obstruction and other symptoms was detected.

Of all 116 patients, 54 survived (46.6%) with a median survival period of 52 months (ranging from 17 to 69 months). Sixty-two patients (53.4%) died with a median survival period of 13 months (ranging from 0.5 to 63.5 months). The mean value of MVD hot spots for

Table 1. Tumor characteristics of 119 primary colorectal carcinoma patients

Characteristics		Numbers of patients (%)
Age	< 62 years (median = 62)	58 (48.7)
	≥ 62 years	61 (51.3)
Sex	Male	68 (57.1)
	Female	51 (42.9)
Stage	1	9 (7.6)
	2	44 (36.9)
	3	57 (47.9)
	4	9 (7.6)
Grade	Well	18 (15.2)
	Moderate	88 (73.9)
	Poorly	13 (10.9)
Depth of invasion		
	T1	3 (2.5)
	T2	12 (10.1)
	T3	88 (73.9)
	T4	16 (13.5)
Distance metastasis		
	No	111 (93.3)
	Yes	8 (6.7)
Lymphatic vascular invasion		
	No	42 (35.3)
	Yes	77 (64.7)
Perineural invasion		
	No	71 (59.6)
	Yes	48 (40.4)
Symptom		
	Obstruction	59 (49.5)
	Others	60 (50.5)
Location		
	Colon	72 (60.5)
	Rectum	47 (39.5)

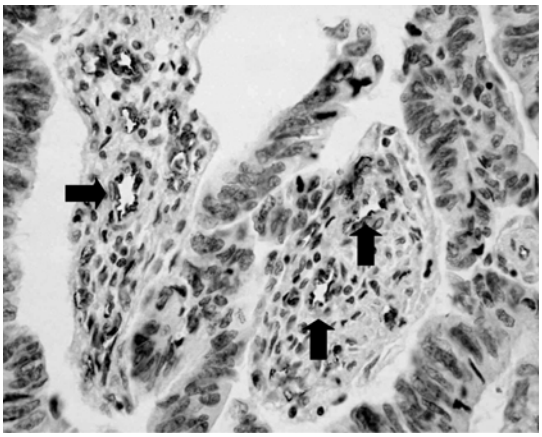

Fig. 1 Example of hot spot area, dispersed stromal microvessels in high magnification (x 400) were stained by CD31 antibody. In this sample the MVD was 24 vessels/HPF (high MVD)

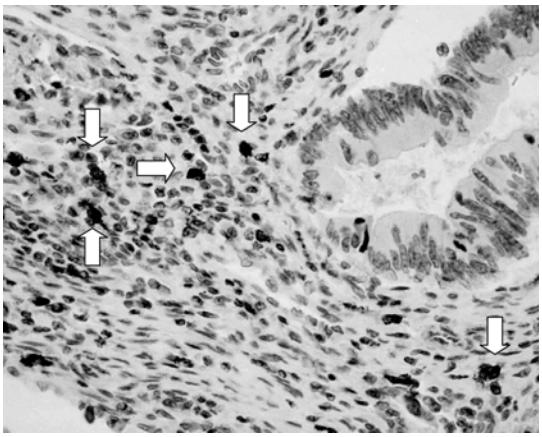

Fig. 2 Example of hot spot area, dispersed stromal mast cells in high magnification (x 400) were stained by mast cell tryptase antibody. In this sample the MCD was 18 cells/HPF (high MCD)

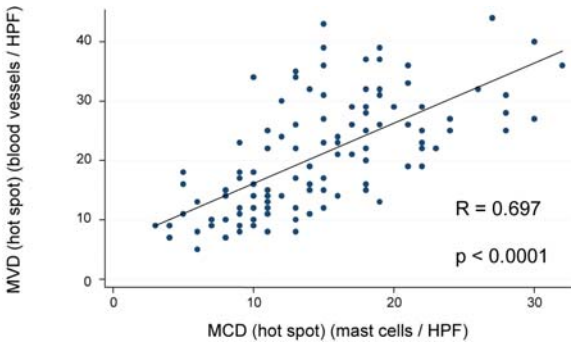

Fig. 3 Positive Spearman rank's correlation of microvessel density (MVD) and tryptase positive mast cell density (MCD) of the hot spot

survivors, however, was significantly lower (16.3 vessels/HPF $\pm$ SD of 8) compared to that of the 62 patients who died before the end of the follow-up period (25.2 vessels/HPF $\pm$ SD of 8.9, $p < 0.001$, unpaired t-test). Analogously, the MCD of the hot spot mean value of the survivor group was significantly lower (10.9 cells/ HPF $\pm$ SD of 4.3) than that of the non-survivors (18.3 cells/HPF $\pm$ SD of 5.5, $p < 0.001$, unpaired t-test).

In a Kaplan-Meier survival estimation, the overall survival was significantly longer for the hypovascular group than the hypervascular group (Fig. 4, log-rank test, $p < 0.0001$). Kaplan-Meier plots of survival in patients with mast cell counts above and below the median of the general study population showed that the high mast cell counts correlated with significantly shorter overall survival rates (Fig. 5, log-rank test, $p < 0.0001$).

To determine the independent prognostic value for survival, microvessel and mast cell densities, as well as other clinicopathological characteristic were included in the univariate analysis. The factors with prognostic significance by log-rank test appeared to be MVD ($p < 0.0001$), MCD ($p < 0.0001$), TNM tumor stage (stage I and II versus stage III and IV) ($p < 0.0001$), distant metastasis ($p < 0.0001$), perineural invasion ($p = 0.0006$), depth of invasion (depth level I and II versus level III and IV) ($p = 0.0058$), lymphatic vascular invasion ($p = 0.0114$) and histological grade (well versus moderate and poor) ($p = 0.0107$). The remaining factors did not reach significant levels. Those composed of symptoms (obstruction versus others) ($p = 0.1006$), age (< 62 versus ≥ 62) ($p = 0.1643$), position (colon versus rectum) ($p = 0.4048$) and gender (male versus female) ($p = 0.9274$). The present study also found a high correlation between MVD and MCD ($R = 0.69$, $p < 0.0001$) (Fig. 3). Therefore, in the fit model of Cox regression utilizing backward stepwise multivariate analysis, avoidance of the problem of multicollinearity was performed by selecting either MVD or MCD into the model. Multicollinearity[41,42] is a situation in which at least some of the independence variables are highly correlated with each other, resulting in inaccurate estimates of the parameter in the regression models. In this case, the authors chose MVD due to its high value for oncological treatment of cancer. Nowadays, the target drug therapy for vessels is developed for destructing vessels, which feed cancer cells in the name of bevacizumab[43]. Multivariate analysis, however, showed that distant metastasis was the strongest independent prognostic factor (Hazard ratio [HR] = 2.54,

Table 2. Clinicalpathological factors associated between microvessel density (MVD) and mast cell density (MCD) in the hot spots of primary colorectal carcinoma (total number = 116)

Variable	Numbers	Hot spots MVD		Hot spots MCD	
		mean	p-value	mean	p-value
Age (median of 62)					
< 62	57	21.3 ± 10.2	0.779*	16.3 ± 6.6	0.433*
≥ 62	59	20.8 ± 9		14.4 ± 5.9	
Sex					
Male	68	20.6 ± 9.9	0.616*	14.2 ± 6.1	0.184*
Female	48	21.5 ± 9.1		15.8 ± 6.3	
TNM staging					
I	9	8.3 ± 2.1	0.0001**	9.4 ± 3.2	0.0001**
II	41	16.5 ± 6.6		11.3 ± 4.4	
III	57	24.8 ± 8.9		17.0 ± 5.6	
IV	9	30.6 ± 7.4		22.8 ± 5.3	
Grade					
Well	18	15.3 ± 9.3	0.0001**	11.7 ± 5.2	0.013**
Moderate	85	21.0 ± 8.9		15.0 ± 6.1	
Poorly	13	28.7 ± 9.5		18.2 ± 6.9	
Depth of invasion					
T1	3	7.3 ± 2.1	0.0001**	9.0 ± 2.7	0.0001**
T2	9	10.2 ± 3.2		8.9 ± 3.4	
T3	88	21.2 ± 9.1		14.6 ± 6	
T4	16	28.6 ± 7.0		19.1 ± 5.7	
Distance metastasis					
No	108	20.4 ± 9.4	0.006*	14.3 ± 6.0	0.001*
Yes	8	29.9 ± 7.6		21.6 ± 4.3	
Lymphatic vascular invasion					
No	37	16.7 ± 8.7	0.001*	12.1 ± 4.4	0.0001*
Yes	79	23.1 ± 9.3		16.1 ± 6.5	
Perineural invasion					
No	68	18.7 ± 9.0	0.002*	13.0 ± 5.8	0.0001*
Yes	48	24.3 ± 9.4		17.3 ± 5.7	
Symptom					
Obstruction	57	23.7 ± 9.4	0.003*	15.3 ± 6	0.469*
Others	59	18.4 ± 9.0		14.4 ± 6.4	
Position					
Rectum	47	21.5 ± 9.2	0.657*	21.5 ± 9.2	0.095*
Colon	69	20.7 ± 9.8		20.7 ± 9.8	

Parametric * t-test, ** Anova test, ☆ significant difference
p-value < 0.05 indicates significant difference

p = 0.024) while MVD was the second-most independent prognostic factor for overall survival (HR = 1.94 p = 0.036) as displayed in Table 3.

Discussion

In the present study, the authors aimed to investigate the role of vascularity and mast cell density as prognostic factors in colorectal carcinoma. For evaluation of tumor vascularity, a monoclonal antibody was used against CD 31 (PECAM-1) along with an adhesion molecule highly expressed in endothelial cell for stain medium vessels. At present, CD 31 is considered the most sensitive pan-specific marker of endothelium cells[31]. In contrast to tumor-associated vasculature in CA breasts where hot spots of angiogenesis are distributed throughout the tumor[32], the hot spots in colorectal cancer may be focal and invasive front[28]. Hence, in the authors' study, the colonic wall

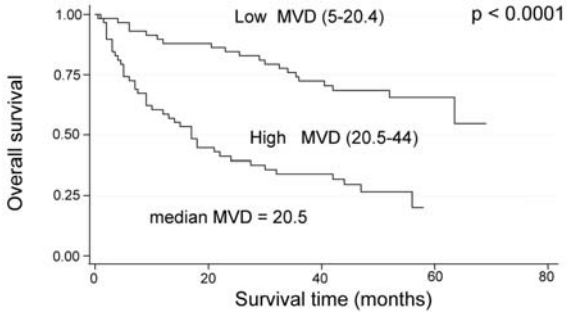

Fig. 4 Relationship between microvessel density (MVD) and survival

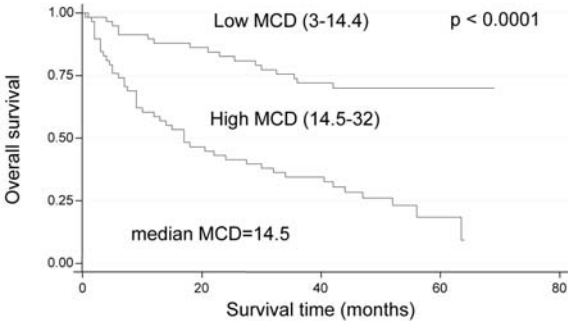

Fig. 5 Relationship between mast cell density (MCD) and survival

Table 3. The multivariate Cox regression analysis for survival in primary colorectal carcinoma patients (total number = 116)

Factor	Hazard ratio	(95% CI)	p-value
Grade	2.056	(0.73-5.79)	0.173
Lymphatic vascular invasion	1.124	(0.55-2.29)	0.777
Depth of invasion	2.646	(0.33-21.1)	0.358
Perineural invasion	1.267	(0.68-2.11)	0.457
Distance metastasis	2.54	(0.96-4.29)	0.024
Stage	2.027	(0.96-4.29)	0.065
Microvessel density	1.942	(1.05-3.61)	0.036

was cut transversally to assess the deepest area of the tumor, called "hot spot" which represents highest intensity of vessels at the invasive front.

Numerous studies have shown that the intratumoral neovascularization has significantly predicted clinical outcomes in many tumors including tumors encountered in lung adenomatous cancer[33], esophagus cancer[34] and cervical cancer[35]. The findings of the current study concurred with previous results, thus confirming the significance of microvessel density as an independent factor for the prognosis of colorectal carcinomas. However, these results were contrary to some studies that revealed no such correlation[36], or an opposing association between MVD and survival[37]. This discrepancy was unclear, thus suggesting that different MVD counting procedures may not be standardized, creating differences in immunohistochemistry antibodies for detected microvessels. Some previous studies (Acikalin et al)[28] reported higher microvessel counts (median of 28) than the results obtained in the study (median of 20.5). This difference may be explained by different uses of vessel markers and different counting methodologies.

The findings of the present study also indicated a strongly significant positive linear correlation between hot spots, MVD and MCD. These results supported the view that mast cells could have possibly played a role in tumor-associated angiogenesis. The authors also showed a significant correlation between low MCD and longer survival rates, which was similar to other studies[28,41]. Therefore, increasing mast cell counts in colorectal carcinoma is correlated with the worse prognosis. However, some studies reported higher mast cell counts were related to favorable prognosis in lung carcinoma[42] and breast carcinoma[43]. This discrepancy in the results might be due to different immunohistochemistry stains for mast cells. For example, in the study of carcinomas of the cervix, using mast cells stained by toluidine blue might have decreased the numbers of the counted mast cell compared to using tryptase positive mast cell stain[35].

In conclusion, most of the patients with primary colorectal carcinoma in the present study were males in the older age group ($\geq$ 62 years old), who had been predominately diagnosed with colonic tumor in the advance stage (stage III) with poor differentiated grades, more depth of invasion (T3-T4), mainly perineural and lymphatic vascular invasion and mostly with symptoms of obstruction and scarce distant metastasis. The immunohistochemistry study yielded

strongly significant positive linear correlations between hot spots, MVD and MCD. Moreover, the survival analysis revealed higher MVD and higher MCD at the invasive fronts of the tumor groups were significantly associated with decreased survival for the patients. The same was true for high TNM staging, high grade, more depth of invasion, presence of lymphatic vascular invasion and perineural invasion, whereas only high MVD and distant metastasis were found to be independent prognostic factors following the control confounding factors by backward stepwise multivariate proportional Cox's hazard analysis. The additional detections of the present study during the period from January 2002 to September 2007 included high MVD patients with a death rate 1.9 times higher than low MVD groups. Whereas, the distant metastasis group had a death rate 2.5 times higher than the non metastasis group in the same duration follow-up period.

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ความสำคัญในการพยากรณ์โรคมะเร็งลำไส้ใหญ่ในคนไทยโดยใช้ค่าความหนาแน่นของหลอดเลือดขนาดเล็ก และความหนาแน่นของแมสต์เซลล์

สิริสรรพางค์ ยอดอาวุธ, ศิริวรรณ ตั้งจิตกมล, สุภลาภ พวงสะอาด

***ภูมิหลัง**: การสร้างหลอดเลือดใหม่จัดว่าเป็นดัชนีบ่งชี้ความร้ายแรงของโรคที่น่าเชื่อถือได้ในมะเร็งหลายชนิด อย่างไรก็ตาม สำหรับมะเร็งลำไส้ใหญ่ ผลการศึกษายังไม่พบข้อสรุปชัดเจน*

***วัตถุประสงค์**: เพื่อหาความสัมพันธ์ระหว่างความหนาแน่นของหลอดเลือดขนาดเล็กและความหนาแน่นของแมสต์เซลล์ และประเมินปัจจัยทั้งสองในการพยากรณ์โรคของผู้ป่วยมะเร็งลำไส้ใหญ่ในคนไทย*

***วัสดุและวิธีการ**: ผู้ป่วยที่ได้รับการวินิจฉัยเป็นมะเร็งลำไส้ใหญ่จำนวน 130 ราย ระหว่างเดือนมกราคม พ.ศ. 2545 ถึงเดือนธันวาคม พ.ศ. 2547 ผู้ป่วย 11 รายถูกคัดออกเนื่องจากมีการกลับเป็นซ้ำของมะเร็ง 8 ราย และไม่พบบล็อกชิ้นเนื้อ 3 ราย เหลือ 119 ราย ที่ได้รับการคัดเลือกเข้าศึกษา ชิ้นเนื้อมะเร็งบริเวณที่มีความหนาแน่นของหลอดเลือดสูงถูกนำมาศึกษาโดยเทคนิค tissue micro-array (เรียง9x5แถวต่อหนึ่งสไลด์) สไลด์ถูกนำมาย้อมสีอิมมูโน anti CD 31 เพื่อนับหลอดเลือดขนาดเล็กและย้อมสีอิมมูโนแอนติบอดี mast cell tryptase เพื่อนับแมสต์เซลล์ ตรวจนับสามครั้งในเวลาที่แตกต่างกันภายใต้กำลังขยายสูงสุดโดยไม่ทราบข้อมูลผู้ป่วย บันทึกค่าสูงสุดของจำนวนหลอดเลือดและแมสต์เซลล์ที่นับได้เป็นค่า MVD และ MCD ผู้ป่วยถูกจัดเป็นกลุ่มความหนาแน่นของหลอดเลือดสูงและความหนาแน่นของแมสต์เซลล์สูง และกลุ่มความหนาแน่นของหลอดเลือดต่ำและความหนาแน่นของแมสต์เซลล์ต่ำโดยอาศัยค่ามัธยฐานของหลอดเลือด (20.5) และแมสต์เซลล์ (14.5) ศึกษาอัตราการรอดชีวิตโดยวิธี Kaplan-Meier และ วิเคราะห์หาปัจจัยที่มีความสำคัญในการพยากรณ์โรคโดยวิธี Multivariate Cox regression*

***ผลการศึกษา**: พบความสัมพันธ์อย่างมีนัยสำคัญทางสถิติระหว่างความหนาแน่นของหลอดเลือดขนาดเล็กและแมสต์เซลล์ ในแง่ของปัจจัยการพยากรณ์โรคพบว่ากลุ่มความหนาแน่นหลอดเลือดต่ำและกลุ่มความหนาแน่นแมสต์เซลล์ต่ำ มีระยะเวลาการรอดชีวิตนานกว่ากลุ่มความหนาแน่นหลอดเลือดสูงและกลุ่มความหนาแน่นแมสต์เซลล์สูง ความหนาแน่นของหลอดเลือดและการแพร่กระจายของมะเร็งเป็นปัจจัยที่ใช้พยากรณ์การรอดชีวิตที่สำคัญ อัตราการตายของกลุ่มความหนาแน่นหลอดเลือดสูงและกลุ่มที่มะเร็งแพร่กระจายสูงเป็น 1.9 และ 2.5 เท่ามากกว่ากลุ่มความหนาแน่นหลอดเลือดต่ำและกลุ่มที่มะเร็งยังไม่แพร่กระจายตามลำดับในช่วงเวลาศึกษาตั้งแต่เดือนมกราคม พ.ศ. 2545 ถึง เดือนกันยายน พ.ศ. 2550*

***สรุป**: การศึกษาครั้งนี้พบว่าความหนาแน่นของหลอดเลือดขนาดเล็กสามารถนำมาใช้เป็นปัจจัยในการพยากรณ์โรคมะเร็งลำไส้ใหญ่ในคนไทย*