A comparative study of protein expressions in primary colorectal cancer and synchronous hepatic metastases: The significance of MMP-1 expression as a predictor of liver metastasis

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A comparative study of protein expressions in primary colorectal cancer and synchronous hepatic metastases: The significance of MMP-1 expression as a predictor of liver metastasis

Directed by Professor Nam Kyu Kim

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오늘의 제가 있기까지 모든 사랑과 배려로 저를 키워 주신 부모님과 가족들에게 감사하다는 말을 전합니다. 무엇하나 믿음직스럽지 못한 저를 뒤에서 항상 도와주시고 언제나 저의 버팀목이 되어 힘이 되어 주시는 부모님, 항상 고명 사위한테 큰 사랑과 가르침을 주시는 장모님의 노고에 정말 무한한 감사를 드리고 보다 발전하는 모습을 보여드리는 것이 조그마한 보답이라고 생각하며 항상 열심히 맡은 일에 전념 할 것을 약속드립니다.

마지막으로 힘든 생활 속에서도 오직 저 하나만을 믿고 저를 위해 희생해가며 저의 뒷바라지를 위해 힘쓰는 저의 소중한 아내에게 무한한 존경과 감사를 전하며 저의 작은 결실인 이 논문의 기쁨을 같이 하려고 합니다.

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<ABSTRACT>

A comparative study of protein expressions in primary colorectal cancer and synchronous hepatic metastases: The significance of MMP-1 expression as a predictor of liver metastasis

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Objective: This study was undertaken to determine the ability of protein expression in primary colorectal cancer and its metastatic liver tumour tissues to predict hepatic metastasis and intrahepatic recurrence.

Materials and Methods: A total of 60 patients with colorectal cancer were enrolled in this study. The expressions of 5 proteins, carcinoembryonic antigen (CEA), vascular endothelial growth factor (VEGF), matrix metalloproteinase (MMP)-1, MMP-7, and tissue inhibitor of metalloproteinases (TIMP)-1, were assessed by immunohistochemical (IHC) staining. Protein expressions were measured in primary colorectal cancer without liver metastasis

(group A), in primary colorectal cancer with liver metastasis (primary tumour, group B), and in resected metastatic liver tumour tissues (liver metastasis, group C).

Results: IHC staining revealed more protease activity (MMP-1 and MMP-7) in group B than in group A. Angiogenic activity, positive VEGF expression, was significantly greater in group C than in group B. Multivariate analysis showed that positive MMP-1 expression, the presence of lymphovascular invasion, and elevated preoperative serum CEA level(>5ng/ml) were significantly related to synchronous liver metastasis. However, intrahepatic recurrence was not found to be related to protein expressions, the presence of lymphovascular invasion, or preoperative CEA level.

Conclusions: Our findings suggest that protease activity is important for metastasis, and that angiogenic activity is essential for metastatic tumour growth. Furthermore, positive MMP-1 expression in primary colorectal tumour tissues was found to be a significant predictor of liver metastasis. However, the prognostic impact of protein marker expression in terms of intrahepatic recurrence appears to be minimal.

Key words :Colonic Neoplasm, Liver metastasis, Protein expression

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I. INTRODUCTION

In colorectal cancer, the pattern of metastasis to the liver is a complex, multistep process that involves the accumulation of many genetic mutations. More specifically, the metastatic process is known to involve; oncogene and tumour suppressor gene mutations, neovascularization, extracellular matrix degradation, adhesion molecules, growth factors, and enzymes.[1,2]

The first step of metastasis involves the destruction of adjacent supporting structures by metastatic cells, which then enter the circulation system. Successful invasion then depends on the corruption of the host immune system, the infiltration of parenchymal tissues, and subsequent adherence and growth, which depend on a continued ability to survive the host's immune system and the establishment of a blood supply. Thus, the processes

involved are complex and require the temporary or permanent genetic alterations.[3,4]

Carcinoembryonic antigen (CEA) is known to affect metastasis due to its association with cellular adhesion mechanism, whereas vascular endothelial growth factor (VEGF) is a well known angiogenic marker. On the other hand, matrix metalloproteinase (MMP)-1 and MMP-7, and tissue inhibitor of metalloproteinases (TIMP)-1 regulate the proteolysis of local extracellular matrix attachments.[5.6]

To date, few studies have compared primary tumors and their metastatic liver tumors by examining relations between protein expressions and the development of liver metastasis or the effects of these expressions on prognosis. Thus, in the present study, we chose five molecules, namely, CEA, VEGF, MMP-1, MMP-7, and TIMP-1, and we investigated relations between their expressions in primary colorectal cancer tissues and metastatic liver tumour tissues and the development of liver metastasis and intrahepatic recurrence.

II. MATERIALS AND METHODS

Patients

A total of 60 patients that underwent curative surgical resection for primary colorectal cancer without synchronous liver metastasis

(n=31) or with synchronous liver metastasis (n=29) were enrolled in this study between August 2004 and March 2007 at the Department of Surgery, Yonsei University College of Medicine. Curative surgical resection with or without intraoperative radiofrequency ablation was performed for patients with liver metastasis. The protein expressions of CEA, VEGF, MMP-1, MMP-7, and TIMP-1 was measured in primary colorectal cancer tissues without liver metastasis (group A), and in primary colorectal cancer tissues with liver metastasis (primary tumour, group B), and in their surgically excised liver specimens (liver metastasis, group C). This study was approved by the review board at our institute (No. 4-2009-0280)

All 60 patients were registered in a prospective colorectal database and underwent follow-up after surgery, which was conducted until death or the 30thJuly,2009. Two patients(3.3%) were lost to follow-up.

The median follow-up period was 26.8months(range,1−59months).

We chose five well known molecular marker proteins, which are known to be involved in the hepatic metastatic cascade. In detail, MMP-1, MMP-7, and TIMP-1 are associated with extracellular matrix proteolysis. CEA affects metastasis during the invasion stage by modulating cellular alteration, and endothelial growth factor (VEGF) is ubiquitously involved in angiogenesis, a critical

requirement of metastasis.

Immunohistochemical Staining

Specimens stored in formalin-fixed and paraffin-embedded tissue blocks were selected. Paraffin-embedded tissues were prepared and sectioned at 5 μ, pretreated with xylene for 10 minutes, and rehydrated using graded alcohol (100%, 90%, 80%). Endogenous peroxidase activity was blocked by treating sections with methanol containing 0.3% hydrogen peroxide for 15 minutes and antigens were retrieved using antigen retrieval buffer (Dako, Carpentaria, CA). The following primary antibodies were then applied:- MMP-1, mouse monoclonal Ab (R&D Systems, Minneapolis) 1:50 dilution,1hour at room temperature; MMP-7, mouse monoclonal Ab (R&D Systems) 1:50 dilution,1hour at room temperature; TIMP-1,mouse monoclonal Ab (Dako) 1:50 dilution, 1 hour at room temperature); VEGF, mouse monoclonal Ab (R&D Systems) 1:50 dilution,1 hour at room temperature; and CEA, mouse monoclonal Ab (SantaCruz,CA) 1:100 dilution, 1 hour at room temperature. Sections were then washed in PBS (phosphate buffered saline). Secondary Antibodies (EnVision DetectionSystems; Dako) were then applied at room temperature for 30minutes and sections were washed in PBS. Diaminobenzidine tetrahydrochloride was used (Dako) for the peroxidase reaction and sections were

counterstained with hematoxylin(Sigma,St.Louis,MO)

Methods of Analysis

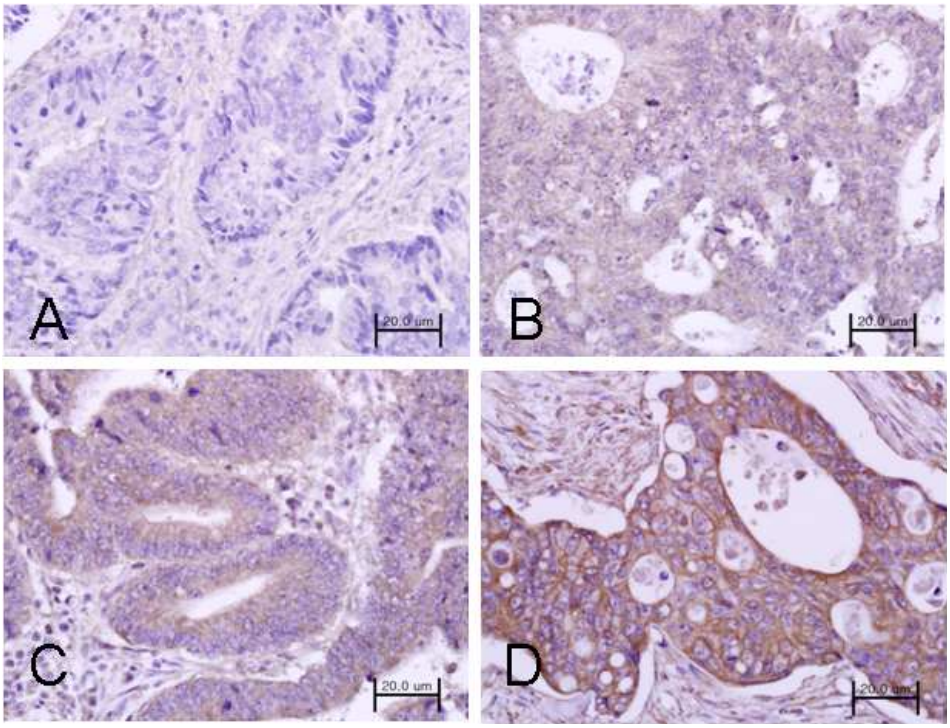

Figure 1. Tumour cell intensities of matrix metalloproteinase-1 (MMP-1) in primary colorectal cancer tissues: A (negative), B (weak), C (moderate), D (strong staining)

Histoscores were classified using a 4-point system: negative (histoscore=0), weak (histoscore 1-100), moderate (histoscore 101-200) and strong (201-300) staining.

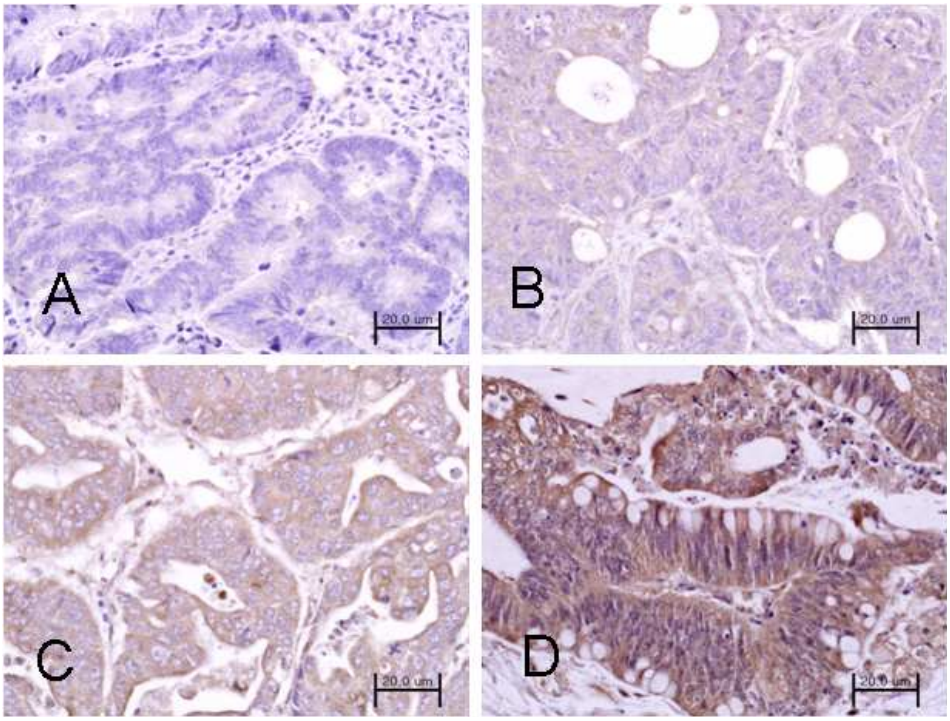

Figure 2. Tumour cell intensities of matrix metalloproteinase-1 (MMP-1) in metastatic liver tumour tissues from colorectal cancer: A (negative), B (weak), C (moderate), D (strong staining)

Histoscores were classified using a 4-point system: negative (histoscore=0), weak (histoscore 1-100), moderate (histoscore 101-200) and strong (201-300) staining.

CEA, MMP-1, MMP-7, and TIMP-1 expressions were evaluated by

examining the cytoplasmic and cell membrane staining of cancer cells (Figures 1 and 2). VEGF expression was determined by evaluating cancer cell cytoplasmic staining.

Two pathologists evaluated all slides using the weighted-histoscore method. Tumour cell intensities were recorded (negative 0, light brown 1, brown 2, dark brown 3). Histoscores were calculated by summing 1 x the percentage of cells stained weakly positive, 2 x the percentage of cells staining moderately positive, and 3 x the percentage of cells staining strongly positive. The maximum possible score was 300. Histoscores were categorized using a 4-point system: negative (histoscore=0), weak (histoscore 1-100), moderate (histoscore 101-200) and strong (201-300). Inter-class correlation coefficients (ICCC) were calculated to determine the consistencies of two observations. [7]

Statistical analysis

Statistical analyses were performed using SAS 9.1.3 Service Pack 4 (SAS Institute Inc., Cary, NC). Categoric variables were analyzed using the chi-squared test or Fisher's exact test. Factors associated with liver metastasis were identified by logistic regression analysis. P values of <0.05 were considered significant.

For statistical analysis, negative and weak protein expressions, as determined using histoscores, were regarded as negative staining.

Thus, only moderate and strong expressions were regarded as positive staining.

III. RESULTS

Patient characteristics

		Group A (n=31)		Group B (n=29)		
		N	%	N	%	P
Gender	Male	21	67.7%	19	65.5%	0.855
	Female	10	32.3%	10	34.5%	
Age	<60	19	61.3%	19	65.5%	0.734
	>60	12	38.7%	10	34.5%	
Location	Right colon	6	19.4%	3	10.3%	0.253
	Left colon	7	22.6%	12	41.4%	
	Rectum	18	58.1%	14	48.3%	
pT	1	1	3.2%	0	.0%	0.144
	2	5	16.1%	1	3.4%	
	3	24	77.4%	24	82.8%	
	4	1	3.2%	4	13.8%	
pN	0	10	32.3%	10	34.5%	0.912
	1	9	29.0%	7	24.1%	
	2	12	38.7%	12	41.4%	
Histology	WD	3	9.7%	3	10.3%	0.259
	MD	23	74.2%	25	86.2%	
	PD	1	3.2%	1	3.4%	
	Mucinous	4	12.9%	0	.0%	
LVI	_	28	90.3%	15	51.7%	0.003
	+	3	9.7%	14	48.3%	
PNI	-	30	96.8%	24	82.8%	0.071
	+	1	3.2%	5	17.2%	
Primary tumor diameter	<4cm	13	41.9%	9	31.0%	0.381
	>4cm	18	58.1%	20	69.0%	
Preoperative CEA	<5ng/ml	26	83.9%	15	51.7%	0.007
	>5ng/ml	5	16.1%	14	48.3%	

Table 1. Patient's characteristics (n=60)

Group A, colorectal cancer patients without liver metastasis; group B, colorectal cancer patients with liver metastasis; WD, well differentiated; MD, moderately

differentiated; PD, poorly differentiated; LVI, lymphovascular invasion; PNI, perineural invasion; CEA, Carcinoembryonic antigen.

Genders, ages, pathologic T and N classifications, histologic grades, and perineural invasion rates were similar in groups A and B. However, lymphovascular invasion (9.7% vs. 48.3%, p=0.003) and an elevated level (>5ng/ml) of preoperative serum CEA (16.1% vs. 48.3%, p=0.007) were more commonly found in group B (Table 1).

Protein expressions of the molecular markers

		Group A (n=31)		B (n=29)			C (n=29)		
		N	%	N	%	P	N	%	P
CEA	-	12	38.7%	2	6.9%	0.004	5	17.2%	0.227
	+	19	61.3%	27	93.1%		24	82.8%	
VEGF	-	26	83.9%	25	86.2%	0.800	16	55.2%	0.009
	+	5	16.1%	4	13.8%		13	44.8%	
MMP-1	-	15	48.4%	3	10.3%	0.001	6	20.7%	0.277
	+	16	51.6%	26	89.7%		23	79.3%	
MMP-7	-	11	35.5%	3	10.3%	0.021	2	6.9%	0.640
	+	20	64.5%	26	89.7%		27	93.1%	
TIMP-1	-	24	77.4%	16	55.2%	0.068	13	44.8%	0.431
	+	7	22.6%	13	44.8%		16	55.2%	

Table 2. Protein expressions of molecular markers by immunohistochemical staining.

P values versus group B.

Group A, primary tumor in colorectal cancer patients without liver metastasis; group B, primary tumor in colorectal cancer patients with liver metastasis; group C, metastatic liver tumor in colorectal cancer patients with liver metastasis; CEA, Carcinoembryonic antigen; VEGF, vascular endothelial growth factor; MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinases.

Immunohistochemical staining revealed more frequent CEA expression (61.3% vs. 93.1%, p=0.004), MMP-1 expression (51.6% vs. 89.7%, p=0.001), and MMP-7 expression (64.5% vs. 89.7%, p=0.021) in group B than in group A. However, VEGF (16.1% vs. 13.8%, p=0.800) and TIMP-1 (22.6% vs. 44.8%, p=0.068) expressions were no different in groups A and B, respectively.

As compared with group B, group C (the resected liver tissues of group B patients) showed more frequent VEGF expression (13.8% vs. 44.8%, p=0.009). However, CEA (93.1% vs. 82.8%, p=0.227), MMP-1 (89.7% vs. 79.3%, p=0.277), MMP-7 (89.7% vs. 93.1%, p=0.640), and TIMP-1 (44.8% vs. 55.2%, p=0.431) expressions were no in these two groups (Table 2).

Factors associated with liver metastasis by multivariate logistic regression

	P	Hazard ratio	95.0% CI
MMP-1 expression (-) vs. (+)	.043	5.409	1.058-27.662
MMP-7 expression (-) vs. (+)	.302	2.491	.441-14.080
LVI (-) vs. (+)	.018	6.532	1.374-31.040
Preoperative CEA (ng/ml) <5 vs. >5	.030	4.754	1.158-19.506

Table 3. Factors associated with liver metastasis by multivariate logistic regression analysis
CI, confidence interval; MMP, matrix metalloproteinase; LVI, lymphovascular invasion; CEA, Carcinoembryonic antigen

Multivariate logistic regression analysis, which included clinicopathological factors and protein expressions showed that the presence of lymphovascular invasion, an elevated preoperative serum CEA level (>5ng/ml), and positivity for MMP-1 expression in immunohistochemical staining, were significantly related to synchronous liver metastasis (Table 3).

Correlation between molecular markers and clinical variables

		CEA +		VEGF +		mmp1 +		mmp7 +		Timp +	
		n	p	n	p	n	p	n	p	n	p
Gende	M	32	.388	7	.443	29	.550	31	.829	15	.333
	14		2		13		15		5		
Age	<60	31	.237	4	.202	26	.726	29	.933	14	.449
	15		5		16		17		6		
Locatio	Rt	5	.047	2	.426	4	.098	4	.013	1	.154
	18		4		16		18		9		
	23		3		22		24		10		
pT	1	0	.061	1	.030	1	.414	0	.170	0	.264
	3		1		4		5		0		
	38		5		32		36		18		
	5		2		5		5		2		
pN	0	15	.933	3	.449	18	.011	14	.453	4	.290
	12		1		7		14		6		
	19		5		17		18		10		
LVI	-	30	.044	6	.718	27	.053	31	.183	12	.156
	16		3		15		15		8		
preCEA_	≤5	28	.024	5	.371	27	.303	30	.347	10	.031
	18		4		15		16		10		

Factors associated with prognosis

		Group B					Group C				
		Intrahepatic recurrence					Intrahepatic recurrence				
		No		Yes			No		Yes		
		N	%	N	%	P	N	%	N	%	P
CEA expression	-	2	10.0%	0	.0%	.326	3	15.0%	2	22.2%	.634
	+	18	90.0%	9	100.0%		17	85.0%	7	77.8%	
VEGF expression	-	17	85.0%	8	88.9%	.779	10	50.0%	6	66.7%	.404
	+	3	15.0%	1	11.1%		10	50.0%	3	33.3%	
MMP-1 expression	-	3	15.0%	0	.0%	.220	3	15.0%	3	33.3%	.260
	+	17	85.0%	9	100.0%		17	85.0%	6	66.7%	
MMP-7 expression	-	3	15.0%	0	.0%	.220	1	5.0%	1	11.1%	.548
	+	17	85.0%	9	100.0%		19	95.0%	8	88.9%	
TIMP-1 expression	-	11	55.0%	5	55.6%	.978	7	35.0%	6	66.7%	.113
	+	9	45.0%	4	44.4%		13	65.0%	3	33.3%	
LVI	-	11	55.0%	4	44.4%	.599					
	+	9	45.0%	5	55.6%						
Preoperative CEA (ng/ml)	>5	10	50.0%	5	55.6%	.782					
	<5	10	50.0%	4	44.4%						

Table 4. Factors associated with intrahepatic recurrence

Group B, primary tumor in colorectal cancer patients with liver metastasis; group C, metastatic liver tumor in colorectal cancer patients with liver metastasis; CEA, Carcinoembryonic antigen; VEGF, Vascular endothelial growth factor; MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinases; LVI,lymphovascular invasion.

The protein expressions of CEA, VEGF, MMP-1, MMP-7, and TIMP-1 in immunohistochemical staining, lymphovascular invasion, and preoperative serum CEA levels were analyzed to determine whether they were associated with prognosis in terms of recurrence. Systemic recurrence and local recurrence were not found to be related to protein expressions, lymphovascular invasion, or preoperative CEA levels (data not shown). Similarly, intrahepatic

recurrence was not found to be associated with protein expression or these clinicopathological factors (Table 4).

IV. DISCUSSION

We undertook this study to identify factors related to synchronous liver metastasis from colorectal cancer. It was found that the presence of lymphovascular invasion, an elevated serum CEA level preoperatively (>5ng/ml), and immunohistochemical positivity for MMP-1 in primary cancer tissues significantly predicted the presence of liver metastasis.

Adachi et al.[8] investigated histopathologic factors associated with liver metastasis in colorectal cancer, and concluded that serosal and venous invasion and lymph node metastasis significantly contribute to liver metastasis. Increased preoperative serum CEA levels have been previously associated with an advanced disease status, an increased risk of recurrence, and poor prognosis.[9,10] Furthermore, Shiozawa et al.[11] demonstrated that MMP-1 immunoreactivity in colorectal carcinoma is significantly associated with the Dukes' class and hepatic metastasis.

In the presence of metastasis to the liver, even after radical hepatectomy, approximately 40 – 50 % of advanced colorectal cases experience recurrence in the liver or at extrahepatic sites. In terms of long-term survival after surgical resection of colorectal metastasis to the liver, the 5-year survival rate has been reported to be 30-40%. Re-resection of intrahepatic recurrence has also been reported to benefit survival. The clinico-pathologic prognostic

factors of long term survival after hepatectomy in such cases have been reported to be; primary cancer location, the sizes and numbers of metastatic tumours, bilateral vs. unilateral, synchronous vs. metachronous, numbers of lymph node metastases, venous invasion, and resection margin status.[12,13,14] Furthermore, clinicopathologic prognostic factors have also been investigated after hepatectomy, but have been reported to depend on investigators, and thus, an examination of the biological characteristics of metastatic tumours might yield an additional means of predicting prognosis. In this study, we evaluated relations between the expressions of protein markers in primary tumour and metastatic tissues on intrahepatic recurrence and local and systemic recurrence. However, we failed to identify any significant relation between the expressions of these protein markers and clinical factors and recurrence. These results could be due to the small size of our cohort or to the inclusion of only five protein molecules. Further study in a larger population and the incorporation of more protein markers might identify relevant prognostic factors after hepatic resection for colorectal liver metastasis.

In present study, we compared protein expressions using three types of tumour tissue samples, namely, from primary colorectal cancers without liver metastasis (group A), from primary colon cancers with liver metastasis (group B), and from metastatic liver

tumour tissues (group C). Interestingly, group B showed more frequent CEA, MMP-1, and MMP-7 expressions than group A, and more frequent serum CEA elevation (>5ng/ml), and this latter finding was found to be well correlated CEA immunohistochemical staining findings. Regarding the mechanism of CEA involvement in metastasis, it has been proposed that it acts as an adhesion molecule by attaching to hepatic vascular adhesion molecules via sLe(α) and sLe(x) (adhesion mechanism), and the mechanism that cytokines released during the process of Kupffer cells attaching to CEA influence the alteration of sinusoid environment. Thereby, sequential reactions of adhesion molecules occur and tumour cells obtain survival capacity (non-adhesion mechanism).[15,16]

According to our MMP-1 and MMP-7 expression results, proteolytic activity is likely to have been significantly greater in group B than in group A. The MMP family is composed of 25 Zn-dependent secretory proteolytic enzymes, and are classified based on their substrate specificities, as interstitial collagenases, stromelysins, gelatinases, elastases, membrane-type proteins, and others. Furthermore, the MMPs have been reported to play decisive roles in the differentiation of colorectal cancer, re-modelling, infiltration, and metastasis. At the molecular level MMPs degrade the collagen network of the ECM and basement membrane components, such as, collagen type 4, fibronectin, laminin, and

gelatin. Moreover, MMPs maintain a relationship with their natural inhibitors the TIMPs.[17,18,19] Many studies have been conducted to elucidate differences between MMP distributions in normal and tumour tissues. Nevertheless, few studies have compared their distributions in primary and secondary tumours. In the present study, no MMP expression differences were found between groups B and C, and more than 80% of tissues were positive for MMP-1 and MMP-7 in both groups, which implies that proteolytic activity is a contributor to the development of liver metastasis.

TIMP-1 expression was not significantly different in groups A and B (22.6% vs. 44.8%, p=0.068), although it tended to be expressed more frequently in group B. Furthermore, imbalances between MMP-9 and TIMP-1 have been suggested to be important for adenoma to carcinoma progression, and some investigators have reported that elevated TIMP-1 expression in tumour tissues is associated with a poor prognosis in colorectal cancer.[20,21]

The neovascularization process is considered to be a necessary for primary or metastatic tumour growth beyond 1-2 mm.[22,23] According to the result of a clinical study conducted recently, VEGF is involved in the growth and migration of vascular endothelial cells. Moreover, it has also been shown that VEGF accelerates metastasis by stimulating angiogenesis, and that it is also involved in the proliferation of lymphatic ducts.[24,25] Interestingly, we did not find

difference between VEGF expressions in groupsA and B, but groupC showed significantly more frequent VEGF expression. These results indirectly support the notion that after initial establishment of a metastatic tumour in liver, angiogenic activity is essential for further tumour growth. Relationships between MMP activity and angiogenesis have also been investigated, and in some *in vitro* models, MMP activity has been found to regulate endothelial cell proliferation.[26,27] Pozzietal.[28] studied the relationship between plasma levels of MMP-9 and endothelial cell proliferation and found that MMP-9 inhibition increased tumour vascularization. They concluded that MMP inhibitors might enhance tumour growth by reducing the generation of endothelial cell inhibitors like angiostatin.

Our results indicate that the clinicopathologic factors and biologic characteristics of primary tumour tissues could be used to predict the development of liver metastasis more precisely. Inaddition, the study provides evidence that supports the notion that targeted therapies, such as, those based on blocking VEGF or epidermal growth factor in patients with colorectal liver metastasis with monoclonal antibodies, because VEGF was found to be highly expressed in metastatic liver tumours. Furthermore, MMP-1 and MMP-7 expressions were also found to be upregulated in metastatic tissues, which suggests that the targeting MMP activity and an investigation of the relationship between MMP activity and

angiogenic activity are warranted.

Summarizing, our results suggest that protease activity is important for liver metastasis development, but that after the establishment of a metastatic tumour in liver, angiogenic activity is crucially required for further tumour growth. Furthermore, positive MMP-1 expression in primary colorectal cancer tissues was found to be a significant predictor of the presence of liver metastasis. However, the impact of protein marker expression on prognosis in terms of intrahepatic recurrence appears to beminimal.

V. CONCLUSION

Our findings suggest that protease activity is important for metastasis, and that angiogenic activity is essential for metastatic tumour growth. Furthermore, positive MMP-1 expression in primary colorectal tumour tissues was found to be a significant predictor of liver metastasis. However, the prognostic impact of protein marker expression in terms of intrahepatic recurrence appears to be minimal.

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<ABSTRACT(IN KOREAN)>

원발성 대장직장암과 동시성 간전이에서 단백질 표현 정도에 대한 비교 연구; 간전이 표자자로서 MMP-1의 중요성

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고 용택

본 연구는 원발성 대장직장암과 전이성 간암 조직에서 간전이와 간내 재발을 예측할 수 있는 단백질 표현 정도를 판별하기 위해 진행되었다. 전체 60명의 대장직장암 환자가 본 연구에 포함되었다. Carcinoembryonic antigen (CEA), vascular endothelial growth factor (VEGF), matrix metalloproteinase (MMP)-1, MMP-7, and tissue inhibitor of metalloproteinases (TIMP)-1등 총 다섯 가지 단백질의 표현 정도를 면역 조직 화학 염색법을 통해 측정하였다. 단백질 표현 정도는 간전이가 없는 원발성 대장직장암(Group A), 간전이가 있는 원발성 대장직장암(Group B), 절제된 전이성 간암 조직(Group C)에서 측정하였다. 단백질 분해 효소(MMP-1,MMP-7)에서의 면역 조직 화학 염색 정도가 Group A에서 보다 Group B에서 더 현저하게 나타났다. 혈관 신생능에 대해서 VEGF 표현 정도는 Group B에서 보다 Group C에서 보다 의미있는 결과를 나타냈다. 다변량 분석 결과 MMP-1표현 정도와 림프 혈관 전이 여부, 수술전 혈중 CEA 수치 상승 등이 동시성 간전이 여부와 의미있게 연관된 인자들로 나타났다. 그러나 간내 재발 유무는 단백질 표현 정도와 림프 혈관 전이 여부, 수술전 CEA 수치와는 연관이 없는 것으로 나타났다. 본 연구 결과 단백질 분해 효소 활성도는 전이에 있어서 중요한 요소이며 혈관 신생 활성도는 전이성 종양의 성장에 필수적임을 나타냈다. 또한 원발성 대장직장암 조직에 있어서 MMP-1 단백질의 양성 표현성은 간전이를 예측하는 의미있는 표지자로 밝혀졌다. 그러나 간내 재발에 있어서의 단백질 표지자의 표현성이 예후에 미치는 영향력은 뚜렷하게 증명되지 않았다.

핵심되는 말 : 대장 신생물, 간전이, 단백질 표현성