Expression of Monocarboxylate Transporter (MCT)-4 in Colorectal Cancer and its Role: MCT4 Contributes to the Growth of Colorectal Cancer with Vascular Endothelial Growth Factor

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Abstract. *Background: In tumor cells, monocarboxylate transporter (MCT)-4 regulates the excretion of lactate produced by glycolysis from the cell. MCT4 has also been reported to be involved in tumor growth and infiltration. Similarly, vascular endothelial growth factor (VEGF) is known to be involved in the growth, infiltration, and metastasis of tumors. In this study, we clinically evaluated the relationship between MCT4 and VEGF in colorectal cancer. Materials and Methods: A prospective study was conducted in 210 patients with colorectal cancer who underwent surgical treatment. The clinicopathological data were correlated with the expression of MCT4 and VEGF obtained from immunohistochemical analysis. Results: MCT4 and VEGF were expressed in tumors of 102 (49%) and 129 (61%) patients, respectively. A maximum tumor diameter of 45 mm or more ($p<0.0001$) and a tumor invasion depth of T1 or less ($p<0.0119$) were factors independently correlated with the expression of MCT4 and VEGF, respectively. The tumor size was significantly smaller ($p=0.0031$), and the disease was significantly less advanced ($p=0.0017$), in MCT4-negative/VEGF-positive than MCT4-positive/VEGF-negative cases. Conclusion: We suspect that in colorectal cancer, VEGF is involved in the early stages of tumor growth and MCT4 expression appears as the tumor enlarges and contributes to its further infiltration and growth.*

Monocarboxylate transporter (MCT) is a protein present in the cell membrane and necessary in the metabolic pathway of lactate for its passage through the cell membrane (1). Lactate is produced in many tumor cells and is transported in and out of them in glycolysis and glyconeogenesis. The intracellular pH is regulated as the influx and efflux of lactate are controlled by MCT (Figure 1) (2). Glucose is also converted to lactate and excreted from the cell in a hypoxic environment. If lactate is not excreted, the intracellular lactate concentration increases, and the pH decreases (2, 3). Since apoptosis is induced when the cell becomes acidotic, MCT is considered to excrete lactate to avoid phenomenon (4).

Adenosine triphosphate (ATP) produced by glycolysis is an important material for the body. In tumor cells, the supply of ATP largely depends on glycolysis (5-7). Therefore, for carcinoma cells to survive by avoiding apoptosis, the control of lactate in glycolysis is considered necessary, and MCT is considered to play an important role in this process.

Fourteen families of MCTs have been identified to date. MCT1 and 2 have been shown to be involved in lactate uptake and oxidation, and MCT4 in lactate excretion (2, 8). Végran *et al*. reported that lactate excretion by MCT4 promotes angiogenesis in tumor cells (9). MCT4 has also been suggested to be induced by hypoxia (10, 11). Vascular endothelial growth factor (VEGF) is a well-known growth factor induced by angiogenesis and hypoxia.

From these observations, MCT4 is expected to be involved in the growth and infiltration of colorectal cancer along with VEGF. In this study, the clinical roles of MCT4 and VEGF were evaluated based on their expression in colorectal cancer tissue.

Materials and Methods

Patients and tissue samples. A prospective study was conducted in 210 patients with colorectal cancer who underwent curative resection at the Kurume University Hospital Japan, from January 2002 to December 2004. Cases with synchronous and multiple cancers were excluded from this study. Written informed consent was given by all

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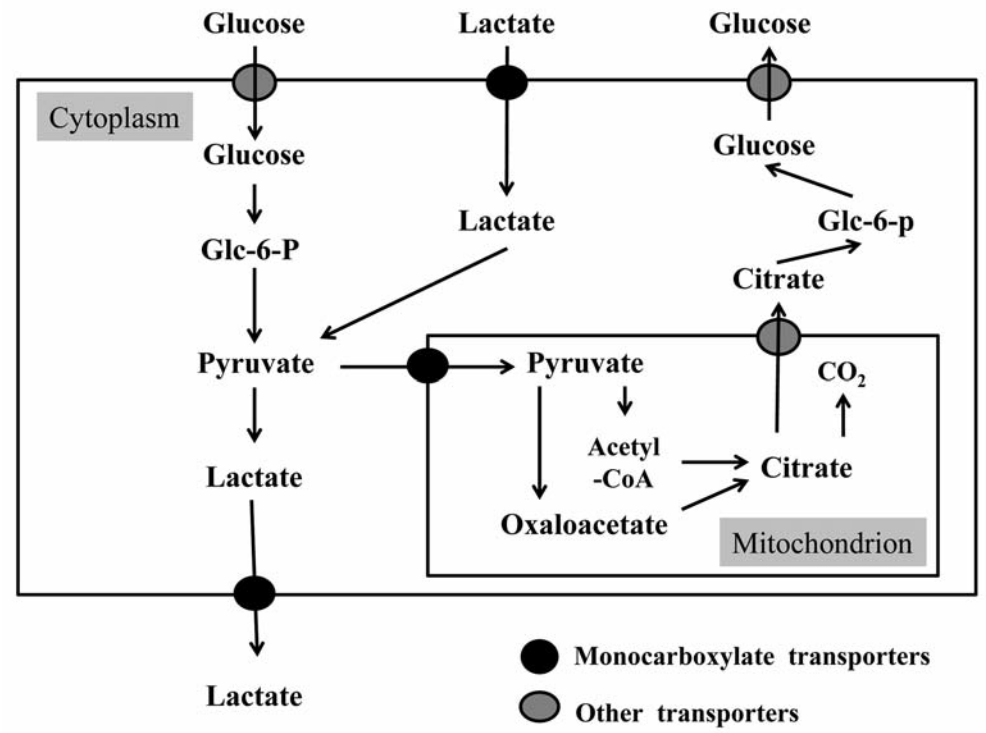


Figure 1. *Localization and activity of Monocarboxylate transporter. Modified from Andrew et al. Biochem J 343: 281-299, 1999.*

patients and ethical approval was obtained from the Ethical Review Board, Kurume University (Approval number 12338). Tumor tissues were acquired from formalin-fixed pathological samples taken from the resected colorectal cancer specimens. Clinicopathological characteristics of patients are presented in Table I. The median age was 67 years, and the median follow-up period was 59.5 months.

Immunohistochemical staining technique. The expression of MCT4 and VEGF in tissue was studied by immunohistochemical staining. From the tissue samples, the section of the tumor with maximal invasion was chosen, cut at 4 μm, examined on a coated slide glass, and labeled with the following antibodies using the BenchMark XT (Ventana Automated Systems, Inc, Tucson, AZ, USA). MCT4 (×150, sc-50329, Santa Cruz Biotechnology, Inc., Santa Cruz CA, USA) and VEGF (×200, sc-152, Santa Cruz Biotechnology, Inc.) were identified with the iVIEW DAB detection kit (Ventana Medical Systems, Inc, Tucson, Arizona, USA) employing the streptavidin biotin complex method and Ultra View universal DAB detection kit (Ventana Medical Systems, Inc, Tucson, Arizona, USA) with the multimer method, respectively. Each slide was heat-treated using Ventana's CC1 retrieval solution for 30 min, and incubated with antibodies for 30 min. The slides were visualized using 3, 3'-diaminobenzidine.

Evaluation of immunohistochemical staining status. For the evaluation of MCT4 expression, the intensity and area of staining in the tumor cell membrane were numerically expressed in combination. The staining intensity was classified into negative, weak, moderate, and strong and graded as 0, 1, 2, and 3, respectively (Figure 2). The stained

Table I. *Patient and tumor characteristics*.

Median follow-up (range), months	59.5 (12-95)
Male:female	131:79
Median age (range), years	67 (29-87)
Tumor location	
Colon	133 (64%)
Rectum	77 (37%)
Median tumor size (range), mm	45 (10-128)
CEA (mg/dl)	
<5.0	113 (61%)
≥5.0	72 (39%)
Depth of invasion	
Tis	13 (6%)
T1	17 (9%)
T2	32 (15%)
T3	82 (39%)
T4	66 (31%)
LN metastasis	
Absent	126 (60%)
Present	72 (34%)
Unknown	12 (6%)
Tumor differentiation	
Well	139 (66%)
Other	71 (34%)
Recurrence	
Absent	189 (90%)
Present	21 (10%)

CEA: Carcinoembryonic antigen, LN: lymph node, Other: moderately- and poorly-differentiated adenocarcinoma.

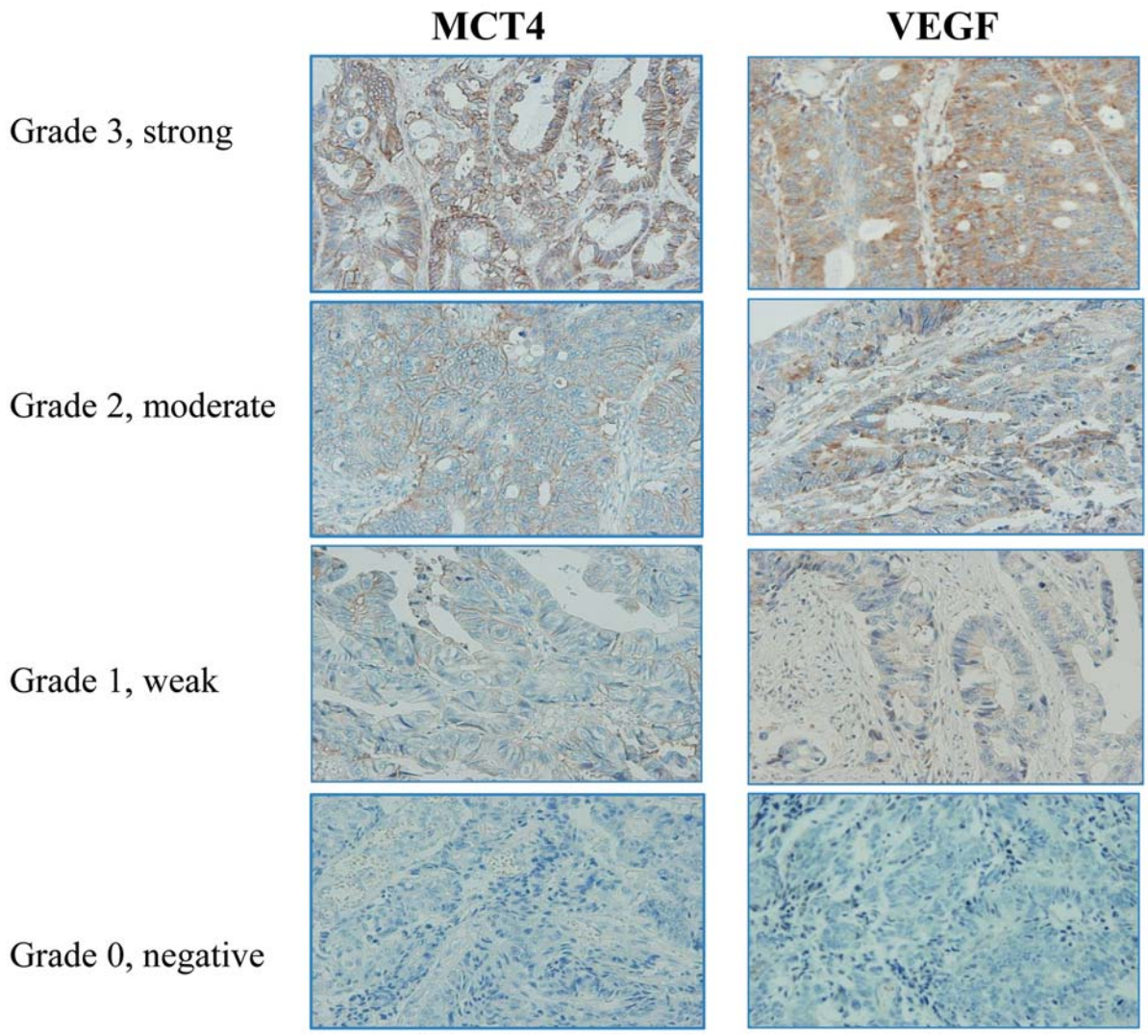


Figure 2. *Intensity of staining for monocarboxylate transporter-4 and vascular endothelial growth factor. Original magnification, ×200.*

area was classified into 0, ≤5, 5-50, and ≥50% and graded as 0, 1, 2, and 3, respectively. In addition, the intensity and area were combined and scored as shown in Table II, and a score of 4 or higher was defined as positive expression (Table II). As for the expression of VEGF, only the staining intensity in the cytoplasm of tumor cells was evaluated alone similarly to the expression of MCT4.

Data analysis. The relationships of the expression of MCT4 and VEGF with clinicopathological factors [gender, age, location, maximum tumor diameter, preoperative carcinoembryonic antigen (CEA), histological type, lymph node metastasis, depth of invasion, lymphatic invasion, venous invasion, and recurrence] were evaluated. The age and maximum tumor diameter were stratified at the median values, and the preoperative CEA by a reference value of 5.0 mg/dl. The degree of MCT4 and VEGF expression was classified by combining them, and their relationships with

Table II. *Classification for immunohistochemical evaluation.*

	Stained area			
	Negative Grade: 0	~5% Grade: 1	5~50% Grade: 2	50%~ Grade: 3
Staining intensity				
Negative Grade: 0	0			
Weak Grade: 1		2	3	4
Moderate Grade: 2		3	4	5
Strong Grade: 3		4	5	6

Table III. *Relationship of Monocarboxylate transporter 4 expression with clinicopathological factors.*

	MCT4 staining+		*p*-Value	Logistic regression analysis		
	Negative (n=108)	Positive (n=102)		Odds ratio	95% CI	*p*-Value
Gender						
Male	71 (54%)	60 (46%)	0.301			
Female	37 (47%)	42 (53%)				
Age (years)						
<67	47 (45%)	57 (55%)	0.0733	0.5813	0.3261~1.0268	0.0617
≥67	61 (58%)	45 (42%)				
Location						
Colon	68 (51%)	65 (49%)	0.9088			
Rectum	40 (52%)	37 (48%)				
Maximum tumor diameter (mm)						
<45	65 (67%)	32 (33%)	<0.0001	3.3931	1.8480~6.3729	<0.0001
≥45	43 (38%)	70 (62%)				
CEA (mg/dl)						
<5.0	61 (54%)	52 (46%)	0.8728			
≥5.0	38 (53%)	34 (47%)				
Tumor differentiation						
Well	70 (50%)	69 (50%)	0.7705			
Other	38 (54%)	33 (46%)				
LN metastasis						
Absent	63 (50%)	63 (50%)	0.5725			
Present	39 (54%)	33 (46%)				
Depth of invasion						
Tis,T1	19 (63%)	11 (37%)	0.1588	0.9812	0.4075~2.4199	0.9812
T2-T4	89 (49%)	91 (51%)				
Lymphatic invasion						
Absent	64 (52%)	58 (48%)	0.725			
Present	44 (50%)	44 (50%)				
Venous invasion						
Absent	29 (48%)	31 (52%)	0.5703			
Present	79 (53%)	71 (47%)				
Recurrence						
Absent	99 (52%)	90 (48%)	0.4074			
Present	9 (43%)	12 (57%)				
Metachronous liver metastasis						
Absent	104 (51%)	98 (49%)	1.0000			
Present	4 (50%)	4 (50%)				

CEA: Carcinoembryonic antigen, CI: confidence interval, LN: lymph node, +score of 4 or more.

clinicopathological factors (maximum tumor diameter, depth, lymph node metastasis, lymphatic invasion, and venous invasion) were evaluated.

Statistical analysis. Statistical analysis was performed using JMP version 9.0 (SAS Institute, Cary, NC, USA). Univariate analysis was carried out using Fisher's exact test, the chi-square test, or the Wilcoxon rank-sum test, depending on the type of data. The observations determined to be significant in the univariate analysis were subsequently subjected to multivariate analysis using logistic regression. A *p*-value of <0.05 was considered significant.

Results

Relationships between MCT4 and VEGF expression and clinicopathological factors. MCT4 and VEGF were expressed in tumors of 102 (49%) and 129 (61%) patients, respectively. On univariate analysis of MCT4 expression, only a maximum tumor diameter of 45 mm or more was observed significantly more frequently in the positive than the negative group. Among the other factors, MCT4 expression might be related to an age of <67 years and a tumor invasion depth of T2 or more. On multivariate analysis using these three factors, only a maximum tumor diameter of 45 mm or more was found to be an independent factor related to MCT4 expression [$p<0.0001$, 95% confidence interval=1.8480-6.3729, Table III).

On univariate analysis of VEGF expression, only a tumor invasion depth of T1 or less was observed significantly more frequently in the positive than the negative group. Among the

Table IV. *Relationship of VEGF expression with clinicopathological factors.*

	VEGF staining		*p*-Value	Logistic regression analysis		
	Negative (n=81)	Positive (n=129)		Odds ratio	95% CI	*p*-Value
Gender						
Male	55 (42%)	76 (58%)	0.1907	1.453	0.8008~2.6728	0.2202
Female	26 (33%)	53 (67%)				
Age (years)						
<67	37 (36%)	67 (64%)	0.3772			
≥67	44 (42%)	62 (58%)				
Location						
Colon	52 (39%)	81 (61%)	0.8368			
Rectum	29 (38%)	48 (62%)				
Maximum tumor diameter (mm)						
<45	38 (39%)	59 (61%)	0.8677			
≥45	43 (38%)	70 (62%)				
CEA (mg/dl)						
<5.0	46 (41%)	67 (59%)	0.6634			
≥5.0	27 (38%)	45 (63%)				
Tumor differentiation						
Well	50 (36%)	89 (64%)	0.2788			
Others	31 (44%)	40 (56%)				
LN metastasis						
Absent	51 (40%)	75 (60%)	0.8698			
Present	30 (42%)	42 (58%)				
Depth of invasion						
Tis,T1	5 (17%)	25 (83%)	0.0078	0.2645	0.0786~0.7555	0.0119
T2-T4	76 (42%)	104 (58%)				
Lymphatic invasion						
Absent	43 (35%)	79 (65%)	0.2437			
Present	38 (43%)	50 (57%)				
Venous invasion						
Absent	19 (32%)	41 (68%)	0.1936	1.0806	0.5166~2.2492	0.8355
Present	62 (41%)	88 (59%)				
Recurrence						
Absent	73 (39%)	116 (61%)	0.9623			
Present	8 (38%)	13 (62%)				
Metachronous liver metastasis						
Absent	78 (39%)	124 (61%)	0.9494			
Present	3 (38%)	5 (63%)				

CEA: Carcinoembryonic antigen, CI: confidence interval, LN : lymph node, +score of 4 or more.

other factors, female gender and venous invasion might be related. On multivariate analysis using these three factors, only tumor invasion depth of T1 or less was found to be an independent factor related to VEGF expression (p=0.0119, 95% CI=0.0119, Table IV). As for the interrelation between MCT4 and VEGF expression, VEGF was expressed significantly more frequently in the MCT4-positive group (p=0.0078, Table V). Therefore, we evaluated patients positive for either MCT4 or VEGF expression to examine the characteristics of their expression. Patients in the MCT4-negative/VEGF-positive group and MCT4-positive/VEGF-negative group were selected, and their clinicopathological characteristics were compared (Table VI). Significant differences were observed in the maximum tumor diameter and tumor invasion depth. These results suggest that MCT4 is expressed more often in large and deep tumors and VEGF is expressed more often in small and shallow tumors.

Discussion

MCT has been reported to be expressed in various cancer types, such as colorectal, breast, stomach, prostatic, lung, and ovarian cancer, and has been suggested to be related to venous invasion, lymph node metastasis, stage, and prognosis (12-17). In particular, MCT4 reportedly increases in cancer cells, promotes their migration and proliferation, and is related to the degree of malignancy and recurrence (9, 16-18). In this study, the expression of MCT4 in colorectal

Table V. *Relationship between (MCT4) and (VEGF) expressions.*

		MCT4			
		Negative	Positive	Total	*p*-Value
VEGF	Negative	51 (47%)	30 (29%)	81	0.0078
	Positive	57 (53%)	72 (71%)	129	
	Total	108	102		

Table VI. *Relationship between (MCT4) and (VEGF).*

	Expression of MCT/VEGF		
	MCT– VEGF+ (n=57)	MCT+ VEGF– (n=30)	*p*-Value
Maximum tumor diameter (mm)			0.0031
<45	35 (61%)	8 (27%)	
≥45	22 (39%)	22 (73%)	
Depth of invasion			0.0017
Tis, T1	14 (25%)	0 (0%)	
T2-T4	43 (75%)	30 (100%)	
LN metastasis			1
Absent	31 (61%)	19 (63%)	
Present	20 (39%)	11 (37%)	
Unknown	6		
Lymphatic invasion			0.4815
Absent	39 (68%)	18 (60%)	
Present	18 (32%)	12 (40%)	
Venous invasion			1
Absent	22 (39%)	12 (40%)	
Present	35 (61%)	18 (60%)	

LN: Lymph node.

cancer tissue was correlated most closely with the maximum tumor diameter (≥45 mm) but was not correlated with the stage or malignancy of cancer. MCT4 was suggested to act primarily as a survival factor in colorectal cancer, *i.e.* it is considered to contribute to the avoidance of apoptosis by promoting lactate excretion from cells.

On the other hand, the expression of VEGF was observed more frequently in early than advanced cancer. Reports to date suggest that VEGF is an adverse prognostic factor (19-24), but it showed no relationship with infiltration or metastasis in this study. This may be explained by the low frequency of metastases and recurrence in our cohort. However, the more frequent expression of VEGF in patients with shallower carcinomas is considered to support the idea that it is involved in an early stage of tumor growth (25). Many colorectal carcinomas are known to develop by a multistage carcinogenic process with the adenoma-carcinoma sequence (ACS) (26). Cancer growth requires angiogenesis to obtain sufficient nutrition, and VEGF plays a particularly important role in this (27, 28). Staton *et al.* suggested the possibility of VEGF involvement in carcinogenesis because its expression was significantly increased in the initial phase of the ACS (29). The results of this study suggest that VEGF promotes angiogenesis and proliferation in an early stage of colorectal carcinogenesis after its onset.

MCT4 and VEGF have been shown to be regulated by a hypoxic environment through hypoxia-inducible factor (HIF-1α) (11). From this, we speculated the following cycle: A hypoxic environment is established first in the development and growth of cancer. VEGF is expressed first and promotes this process. MCT4 is then expressed under the hypoxic conditions in the interior of the enlarged tumor and promotes the continuation of tumor growth.

There have been few reports on the relationship between the expression of MCT4 and clinicopathological factors in colorectal cancer, and the significance of its expression remains largely unclear. In this study, the expression of MCT4 was evaluated with regard to both the intensity and area, an approach considered to be practical for the evaluation of its clinical significance. However, *in vivo* studies are indispensable for discussing the function of MCT. If controlling the expression of MCT is confirmed as suppressing the growth of cancer or promoting apoptosis of cancer cells, its clinical application is further anticipated.

To summarize, MCT4 and VEGF were related to tumor growth and the degree of tumor progression. We speculate that VEGF is involved from an early stage of tumor growth and that the expression of MCT4 is switched on in the process of tumor growth to promote tumor continuation.

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