Expression of angiopoietin-like 4 (ANGPTL4) in human colorectal cancer: ANGPTL4 promotes venous invasion and distant metastasis

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Abstract. There is strong evidence that the angiopoietin family is involved in the regulation of tumour progression. Recently, it has been reported that angiopoietin-like 4 (ANGPTL4) expression in cancer cells promotes the metastatic process by increasing vascular permeability. The present study was conducted to examine ANGPTL4 expression and its association with clinicopathological factors and prognosis in human colorectal cancers. We examined 144 cases of surgically-resected human colorectal adenocarcinomas by immunohistochemistry, RT-PCR and Western blot analysis. Also, overall survival was investigated. Among 144 cases of adenocarcinoma, 95 cases (66.0%) showed positive staining in the cytoplasm of the carcinoma cells for ANGPTL4. Histologically, well, moderately, poorly differentiated adenocarcinoma or mucinous carcinoma showed 55.2, 79.3, 61.5 or 44.4% expression of ANGPTL4, respectively. The expression of ANGPTL4 was correlated with the depth of tumour invasion ($p<0.0005$), Vienna classification (category 3-5) ($p<0.00005$), venous invasion ($p<0.0005$) and Duke's classification ($p<0.005$). However, ANGPTL4 expression was not correlated with overall survival. However, all patients (100%) with distant metastasis showed immunopositivity for ANGPTL4. The mRNA and the protein expression of ANGPTL4 were shown in four resected samples and cultured cell lines by RT-PCR or Western blot analysis. These findings suggest that ANGPTL4 is one of the factors involved in the progression of human colorectal cancer, especially venous invasion and distant metastasis.

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Introduction

Colorectal cancer is one of the most common cancer types in the world today (1). The occurrence and progression of cancer are considered to be a series of genetic events affecting the structure and/or expression of a number of oncogenes, tumour suppressors, and growth factors (2,3). The deep invasive carcinomas, such as colorectal cancer, have higher rates of lymph duct and venous invasions, and lymph node metastasis (3). However, the mechanisms of invasion and metastasis of colorectal cancer are not fully understood.

There is strong evidence that the angiopoietin family is involved in the regulation of tumour progression, cellular growth, and differentiation (4-6). Angiopoietin-like 4 (ANGPTL4) is a member of the family of angiopoietins and is also known as hepatic fibrinogen/angiopoietin-related protein (HEARP) (7), peroxisome proliferator-activated receptor-γ (PPARγ) angiopoietin-related gene (PGAR) (8), or fasting-induced adipose factor (FIAF) (9). ANGPTL4 is a circulating plasma protein and is expressed in the liver, adipose tissue, placenta, as well as in ischemic tissue (8,9). Similar to angiopoietins and other angiopoietin-like proteins, ANGPTL4 contains an amino-terminal coiled-coil domain and a carboxyl-terminal fibrinogen-like domain (10). ANGPTL4 induces a strong proangiogenic response, independently of vascular endothelial growth factor (11), and is known to undergo hypoxia-induced gene expression in endothelial cells. This protein is up-regulated by fasting and peroxisome proliferator-activated receptor agonists, associates with lipoproteins (8), and is involved in regulating glucose homeostasis, insulin sensitivity, and lipid metabolism through its ability to inhibit lipoprotein lipase (8,12-14). However, the role of ANGPTL4 is little known in cancer biology, although the role of ANGPTL4 has been well characterized in ischemic conditions and lipid metabolism.

Recently, it has been reported that the induction of ANGPTL4 by TGF-β primes breast cancer cells for lung metastasis (15). Tumour cell-derived ANGPTL4 disrupts vascular endothelial cell-cell junctions, increases the permeability of lung capillaries, and facilitates the trans-endothelial passage of cancer cells. Secretion of ANGPTL4 enables tumour cells

to extravasate into other tissue and to seed micrometastases. However, it has been also reported that ANGPTL4 prevents the metastatic process by inhibiting vascular activity as well as tumor cell motility and invasiveness (16,17). The effects of ANGPTL4 in experimental systems are still unclear in tumour biology.

The objective of the present study is to evaluate the role of ANGPTL4 in the progression and differentiation of human colorectal carcinoma, especially with regard to migration to vasculature and distant metastasis to other organs.

Materials and methods

Patients. We studied 144 primary human colorectal adenocarcinomas: 30 mucosal carcinomas (pTis), 13 submucosal infiltrative carcinomas (pT1), 10 carcinomas invading proprial muscle layers (pT2), 59 carcinomas invading subserosa (pT3), and 32 carcinomas penetrating serosa or invading adjacent structures (pT4). All tumour specimens were obtained from patients operated at Nagasaki University Hospital between 2001 and 2009. Each tumour was assigned a histological type according to the World Health Organization classification (18), and a depth grading of infiltration according to the International Union Against Cancer (UICC), TNM Classification of Malignant Tumours (19). Mucosal neoplasias were classified by the Vienna classification (20). Histologically, of the 144 primary human colorectal adenocarcinomas, 23 were of the carcinoma in adenoma type, 64 were the well-differentiated type (well), 58 were the moderately differentiated type (moderate), 6 were poorly differentiated adenocarcinomas of the solid type (poor/solid), 7 were poorly differentiated adenocarcinomas of the non-solid type (poor/non-solid), and 9 were mucinous adenocarcinomas (mucinous).

We used 10 adenomas as benign lesions with low or moderate dysplasia and 6 adenomas with high grade dysplasia resected by endoscopic mucosal resection (EMR). Fifteen specimens of normal colon mucosal tissue were evaluated as normal controls. The desmoplastic stromal reaction was graded according to the extent of the stromal area involved. It was defined as 'slight' (when the fibrous stromal area was <25% of the whole tumour), 'moderate' (between 25 and 75%), and 'extensive' (when it exceeded 75% of the whole tumor) based on the overall pattern (21). The examination was performed on routine slides to identify lymphatic and venous invasion. In addition to hematoxylin and eosin staining, we also used elastic van Gieson staining and immunohistochemical staining for CD34 (Dako Ltd., Glostrup, Denmark) and D2-40 (Dako Ltd.) for all cases. Each parameter was defined as 'present' when invasion was identified with certainty, but defined as 'absent' when it was either not observed at all or not observed with certainty (22,23). Lymph node metastasis was defined as 'present' only when histologically proven. Diagnosis was established by two independent pathologists (T.N and H.H), and cases of questionable diagnosis were omitted from the study.

Among the 114 patients with invasive carcinoma, 60 were used for the follow-up study for a median follow-up period of 1627 days, ranging from 22 to 4467 days. Thirty-eight patients remained disease-free, and 25 patients suffered from local recurrence (9 patients), distant metastasis (13 patients) or other cancers (3 patients) after the operation.

Immunohistochemistry. Formalin-fixed and paraffin-embedded tissues were cut into 4-μm sections, deparaffinized in xylene, and rehydrated in phosphate-buffered saline. Deparaffinized sections were preincubated with normal rabbit serum to prevent non-specific binding, and then incubated overnight at 4˚C with an optimal dilution (0.1 μg/ml) of a primary polyclonal goat antibody against human ANGPTL4 (R&D Systems, Inc., Minneapolis, MA, USA). The slides were sequentially incubated with a biotinylated rabbit anti-goat immunoglobulin antibody, and the reaction products were viewed using diaminobenzidine (DAB; Dako Ltd.) and counterstained with hematoxylin. Primary antibody preabsorbed with excess recombinant ANGPTL4 peptides (R&D Systems, Inc.) was used as negative control. Human liver tissue served as the internal positive control for ANGPTL4 immunostaining (8). Analysis of the immunohistochemical staining was performed independently by two investigators (T.N and H.H). ANGPTL4 expression was classified into two categories depending on the percentage of cells stained: -, 0-10% positive cells; +, >10% positive tumour cells.

Cell culture. Caco-2, Colo201, Colo320DM, DLD-1, LS123, and WiDr cell lines, derived from human colorectal cancers, were obtained from American Type Culture Collection (Manassas, VA, USA) (24). Caco-2 was maintained in minimum essential medium (MEM) with 20% FCS. Colo-201 and DLD-1 were maintained in RPMI-1640 with 10% FCS. Colo320DM was maintained in Dulbecco's modified Eagle's medium (DMEM) with 10% FCS. LS123 and WiDr were maintained in EMEM with 10% FCS. All media and fetal calf serum (FCS) were purchased from Invitrogen Corp. (Carlsbad, CA, USA).

All cell lines were supplemented with 2 mM glutamine (Invitrogen Corp.) and incubated at 37˚C in a humidified atmosphere containing 5% CO_2.

Reverse transcription-polymerase chain reaction (RT-PCR. Total RNA was prepared from both cancer tissue and normal mucosa from 3 cases of human colorectal cancer patients, and from the human colorectal carcinoma cell lines, Caco2, Colo201, Colo320DM, DLD-1, LS123, and WiDr (24), using the acid guanidine phenol method (25). Cellular RNA (1 µg) was incubated at 37˚C for 1 h in 50 μl of reverse transcriptase buffer containing 20 units of RNAsin (Promega Corp., Madison, WI, USA), 100 pmol of random hexamer primers (Boehringer Mannheim, Mannheim, Germany), and 400 units of Moloney murine leukemic virus reverse transcriptase (Invitrogen Corp.) Reverse transcription was terminated by heating at 95˚C for 10 min, and 20% of the resultant cDNA was removed for PCR. PCR samples were incubated with 50 pmol of each primer and 2.5 units of Taq DNA polymerase. The human ANGPTL4 PCR primers were 5'-GGCGAGTTCTGGCTGGGTCT-3' (sense) and 5'-TGG CCGTTGAGGTTGGAATG-3' (antisense). The human β-actin PCR primers were 5'-TCCTCCCTGGAGAAGACTA-3' (sense) and 5'-AGTACTTGCGCTCAGGAGGA-3' (antisense). The ANGPTL4 and β-actin primers were predicted to amplify 329 and 313 bp DNA fragments, respectively. Both

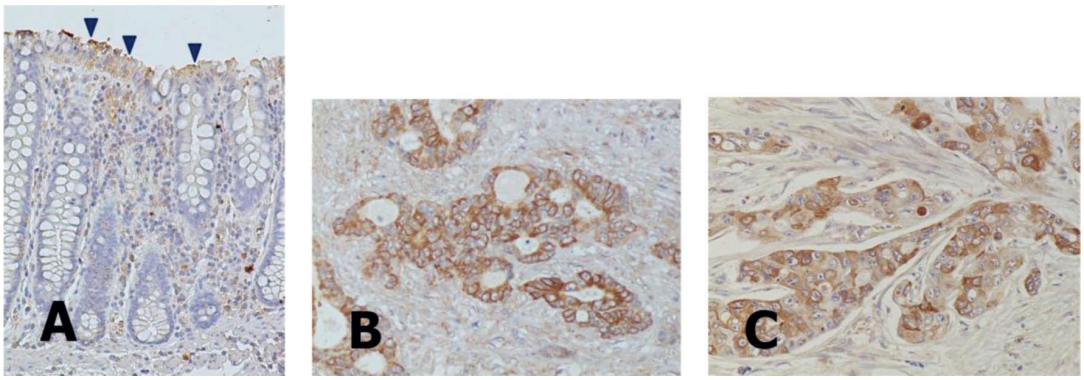

Figure 1. Immunohistochemical staining for ANGPTL4 in human tissues. (A) Faintly expression in the surface lining cells of normal colon mucosa (arrowheads), (B) strongly in cytoplasm of moderately-differentiated adenocarcinoma, and (C) poorly-differentiated adenocarcinoma (non-solid type) of human colorectal cancer (magnification, x100).

primer pairs were chosen to span introns of their respective human genes. Samples were subjected to 30 cycles of PCR amplification using a thermocycler. Each cycle included denaturation at 94˚C for 1 min, annealing at 60˚C for 1 min, and primer extension at 72˚C for 1.5 min. An aliquot of each amplification mixture was subjected to electrophoresis on a 1.5% agarose gel, and DNA was visualized by ethidium bromide staining.

Western blot analysis. Western blot analysis was performed on both cancer tissue and normal mucosa from 3 cases of human colorectal cancer patients, and the 6 cultured cell lines. The tissues obtained at surgery were frozen immediately after tissue sampling. The tissues and the cells were then suspended in RIPA buffer (50 mM Tris, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, and 0.05% SDS, pH 7.4), broken into pieces on ice, and subjected to three freeze-thaw cycles. The insoluble tissue debris was removed by centrifugation at 14,000 × g at 0˚C for 10 min, the supernatant was collected, and the protein concentration was quantified using a protein assay reagent (Bio-Rad Laboratory, Hercules, CA, USA). After boiling, the proteins (20 μg) were separated by polyacrylamide gel electrophoresis (PAGE) under denaturing and reducing conditions, and transferred to Hybond ECL Nitrocellulose Membranes (Amersham Pharmacia Biotech, Arlington Height, IL, USA). The membranes were rinsed in TBS, blocked with 5% low-fat dried milk in TBS containing 0.1% Tween-20 (TBS-T), and then incubated for 1 h at room temperature with a 1:1,000 dilution of the anti-human ANGPTL4 antibody (R&D Systems, Inc.). The anti-human β-actin antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) was used as an indicator for the amount of loaded proteins. After extensive washing with TBS-T, the membranes were incubated for 1 h with a 1:1,000 dilution of the horse-radish-peroxidase-conjugated donkey anti-goat immunoglobulin G for ANGPTL4 or anti-rabbit immunoglobulin G for β-actin (Santa Cruz Biotechnology) in TBS-T containing 3% low-fat dried milk. The membranes were washed and developed with a horseradish peroxidase chemiluminescence detection reagent (ECL Plus System, Amersham Pharmacia Biotech), and then exposed to Hyperfilm ECL (Amersham Pharmacia Biotech).

Statistical analysis. The Stat View II program (Abacus Concepts, Inc., Berkeley, CA, USA) was used for statistical analyses. Analyses comparing the expression of ANGPTL4 were performed by the χ^2 test for independence, Fisher's exact probability test or the Mann-Whitney's U test. Survival durations were calculated using the Kaplan-Meier method. A log-rank test was used to calculate the significance of differences in the survival analysis. A probability level of <0.05 ($p<0.05$) was considered to indicate a significant difference.

Results

Immunohistochemical analyses of clinicopathological factors. In normal colorectal mucosa, ANGPTL4 was faintly expressed in the surface lining cells (Fig. 1A). Further, ANGPTL4 was also expressed faintly in adipose tissue and neural cells of the submucosal Meissner's plexus and myenteric plexus (data not shown).

We have summarized the immunohistochemical results in Tables I and II. Only 1 of 10 (10%) adenoma cases with low or moderate dysplasia (Vienna 3), resected by endoscopic mucosal resection (EMR), had positive staining for ANGPTL4 (Table I). Further, only 1 of 6 (16.7%) cases of Vienna 4.1 were positive for ANGPTL4. However, 13 of 30 (43.3%) cases of carcinoma *in situ* (Vienna 4.2) and 95 of 114 (66.0%) of invasive carcinoma (Vienna 5) showed highly positive staining for ANGPTL4 in the cytoplasm of carcinoma cells (Fig. 1B and C and Table I). Statistical analysis showed significant correlation of ANGPTL4 staining with the Vienna classification from grades 3 to 5 ($p<0.00001$). Also, the invasive component of the primary tumour was more intensely stained than the superficial part of the tumour in almost all cases of invasive carcinoma.

In total carcinoma, moderately differentiated adenocarcinomas showed relatively high expression of ANGPTL4 (79.3%) (Table II). However, only 4 of 9 cases (44.4%) of mucinous carcinoma showed positive staining for ANGPTL4. The expression of ANGPTL4 was not correlated with the degree of histological differentiation in carcinoma. The degree of immunoreactivity appears to be correlated with the degree of tumour invasion (Table II). Statistical analysis

Table I. The expression of ANGPTL4 in adenoma (cases, %).

	n	+	-	P-value
Total tumour	160	97 (60.6)	63 (39.4)	
Vienna 3 (low or moderate grade)	10	1 (10.0)	9 (90.0)	[a]p<0.00001
Vienna 4	36	14 (38.9)	22 (61.1)	
4.1 (high grade)	6	1 (16.7)	5 (83.3)	
4.2 (carcinoma *in situ*)	30	13 (43.3)	17 (56.7)	
Vienna 5 (invasive carcinoma)	114	82 (71.9)	32 (28.1)	

Table II. The expression of ANGPTL4 in colorectal adenocarcinoma (cases, %).

	n	+	-	
Total cancer	144	95 (66.0)	49 (34.0)	P-value
Histological differentiation				
Carcinoma in adenoma	23	11 (47.8)	12 (52.2)	
Well	64	37 (57.8)	27 (42.2)	n.s.
Moderate	58	46 (79.3)	12 (20.7)	
Poor/solid	6	4 (66.7)	2 (33.3)	
Poor/non-solid	7	4 (57.1)	3 (42.9)	
Mucinous	9	4 (44.4)	5 (55.6)	
Depth of tumour invasion pT grade				
is	30	13 (43.3)	17 (56.7)	[a]p<0.000
1	13	3 (23.1)	10 (76.9)	
2	10	7 (70.0)	3 (30.0)	
3	59	47 (79.7)	12 (20.3)	
4	32	25 (78.1)	7 (21.9)	
Lymph node metastasis				
Present	50	35 (70.0)	15 (30.0)	n.s.
Absent	94	60 (63.8)	34 (36.2)	
Lymph duct invasion				
Present	98	72 (73.5)	26 (26.5)	[a]p<0.01
Absent	46	23 (50.0)	23 (50.0)	
Venous invasion				
Present	64	53 (82.8)	11 (17.2)	[a]p<0.0005
Absent	80	42 (52.5)	38 (47.5)	
Dukes classification				
A	52	23 (44.2)	29 (55.8)	[a]p<0.005
B	38	33 (86.8)	5 (13.2)	
C1	26	18 (69.2)	8 (30.8)	
C2	16	10 (62.5)	6 (37.5)	
D	11	10 (90.9)	1 (9.1)	

n.s., not significant.

showed significant correlation of ANGPTL4 expression with the depth of tumour invasion (pT grade) (p<0.0005). Further, ANGPTL4 expression was correlated with the presence of lymph duct invasion (p<0.01) and Dukes classification (p<0.005). In particular, the expression of ANGPTL4 was significantly correlated with venous invasion (p<0.0005).

Table III. The expression of ANGPTL4 in desmoplastic stromal reaction and tumour growth pattern in invasive cancer (cases, %).

	n	+	-	P-value
Invasive cancer	114	82 (71.9)	32 (28.1)	
Desmoplastic stromal reaction				
Medullary	7	3 (42.9)	4 (57.1)	n.s.
Intermediate	81	59 (72.8)	22 (27.2)	
Scirrhous	26	20 (76.9)	6 (23.1)	
Tumour growth pattern				
Solid	15	7 (46.7)	8 (53.3)	n.s.
Intermediate	77	58 (75.3)	19 (24.7)	
Diffuse	22	17 (77.3)	5 (22.7)	

n.s., not significant.

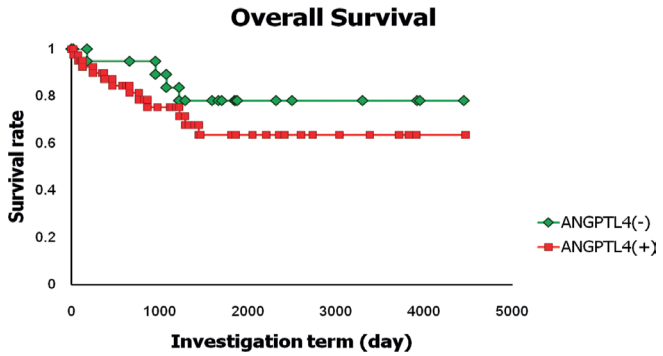

Figure 2. Overall survival based on the expression of ANGPTL4 in human colorectal cancer (60 cases). ANGPTL4 expression was not associated with the overall survival in univariate survival analysis. (p=0.455 in log-rank test).

However, there was no correlation with lymph node metastasis. In 114 cases of invasive cancer, ANGPTL4 expression was not correlated with the desmoplastic stromal reaction or tumour growth pattern (Table III).

Overall survival analysis and prognosis after surgery. We analyzed overall survival in 60 patients with invasive carcinoma based on their expression of ANGPTL4 in human colorectal cancer. However, ANGPTL4 expression was not associated with the overall survival by univariate survival analysis (p=0.455 in log-rank test) (Fig. 2).

ANGPTL4 immunoreactivity was compared with the prognosis after surgery in 60 patients who were also analyzed for overall survival. ANGPTL4 expression was found in 22 of 38 disease-free patients (57.9%) and in 5 of 9 cases (55.6%) with local recurrence (Table IV). The expression of ANGPTL4 was not significantly different between disease-free and local

Table IV. Data of follow-up and the expression of ANGPTL4, (case, %).

	n	+	-	P-value
Total cases	60	40 (66.7)	20 (33.3)	
Disease free	38	22 (57.9)	16 (42.1)	
Distant metastasis	13	13 (100)	0 (0.0)	[a]p<0.005
Local recurrence	9	5 (55.6)	4 (44.4)	n.s.
Other cancer	3	1 (33.3)	2 (66.7)	n.s

n.s., not significant.

recurrence cases. However, all 13 cases with distant metastasis to other organs expressed ANGPTL4: 5 to only liver, 3 to only lung, 4 to liver and lung, and 1 to liver, bone and brain. There was a significant difference between distant metastasis and disease-free survival cases (p<0.005).

RT-PCR for ANGPTL4 in human colorectal tissues and cultured cell lines. RT-PCR showed mRNA expression of ANGPTL4 in 3 tissues of normal colorectal mucosa, 3 of invasive carcinomas, and all 6 cultured cell lines of human colorectal adenocarcinoma (Fig. 3A).

Western blot analysis for ANGPTL4 in human colorectal tissues and cultured cell lines. Western blot analysis showed intense expression of ANGPTL4 protein in 6 surgically-resected samples, 3 normal mucosas, 3 invasive cancers, and 4 of 6 cultured cell lines of human colorectal adenocarcinoma (Fig. 3B). In some patients, ANGPTL4 expression in cancer tissue was more intense than in normal mucosa. Colo320DM, DLD1, WiDr and LS123 showed more intense expression of ANGPTL4 than in Caco 2 and Colo201.

Discussion

The tumour microenvironment plays an important role in molecular mechanism of metastasis (26). Primary carcinomas, as well as metastases, are comprised of both tumour cells and cells of the stroma including fibroblasts, endothelial cells, and inflammatory cells. The cytokine TGF-β is produced by stromal cells of the tumour microenvironment in response to hypoxia or inflammation, or by carcinoma-associated fibroblasts (27). Recently, Padua *et al* showed that TGF-β stimulates expression of the adipokine ANGPTL4 by activating SMAD transcription factors (15). Tumour cell-derived ANGPTL4 disrupts vascular endothelial cell-cell junctions, increases the permeability of capillaries, and facilitates the trans-endothelial passage of tumour cells. Secretion of ANGPTL4 enables tumour cells to extravasate into other tissue and to seed micrometastases. In this study, the expression of ANGPTL4 protein in tissues from all 3 cases of invasive carcinoma in human colorectum was more intense than normal tissue (Fig. 3B). Thus, ANGPTL4 may promote vascular invasion and distant metastasis in human colorectal cancer.

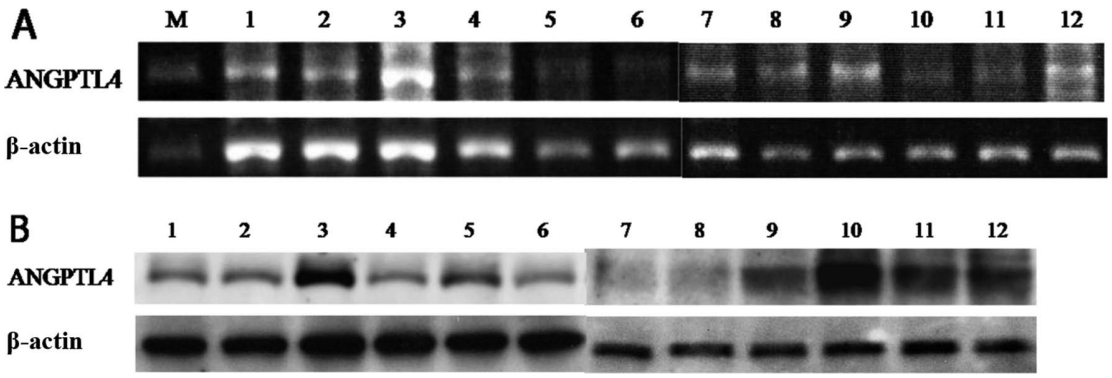

Figure 3. RT-PCR and Western blot analysis for ANGPTL4 in human colo rectal cancer: tissues and cultured cell lines. (A) RT-PCR. M, 100 bp ladder marker (Invitrogen, Inc.). (B) Western blot analysis. Human colorectal cancer tissues (1-6) and human colorectal cancer cell lines (7-12). [1, Cancer tissue; cases 1 and 2, normal gastric tissue; cases 1 and 3, cancer tissue; cases 2 and 4, normal gastric tissue; cases 2, 5, cancer tissue; cases 3 and 6, normal gastric tissue; cases-3 and 7, Caco-2; 8, Colo201; 9, Colo320DM; 10, DLD-1; 11, WiDr,; 12, LS123].

There are some reports on ANGPTL4 protein or gene expression profile in cell lines of breast cancer (15,28), lung cancer (16), hepatocellular carcinoma (29), prostate cancer (30), and oral tongue squamousc cell carcinoma (31). Angiopoietin family members usually regulate the differentiation or invasion of cancer cells through the activation of its receptor Tie-2 and downstream tyrosine kinase pathway (5,6,32). However, ANGPTL4 does not bind to Tie-2, but contain motifs structurally conserved in angiopoietins. The role of ANGPTL4 has not been fully clarified in cancer biology, particularly the relationship between the expression of ANGPTL4 and the clinicopathological features of human cancer. In this study, we investigated the expression of ANGPTL4 in human colorectal cancer using immunohistochemical and molecular biological techniques. This is the first investigation of the role of ANGPTL4 in human colorectal cancer.

Statistical analyses of our data showed a strong correlation between ANGPTL4 expression and venous invasion and lymph duct invasion (Table II). The cancer cells with venous invasion showed strong immunopositivity for ANGPTL4 proteins in the cytoplasm. In our previous study, the expression of ANGPTL4 in gastric cancer was correlated with lymphovascular infiltration (33). One report proposed that ANGPTL4 up-regulates the infiltration of cancer cells into the capillary adjacent to the tumour due to acute disruptions of the endothelial cell-cell junctions caused by ANGPTL4 (15). Further, the strong interaction of ANGPTL4 with the subendothelial extracellular matrix (ECM) is heparin/heparan sulfate proteoglycan-dependent (34). The balance between matrix-associated and soluble forms of ANGPTL4 points to the role of the ECM in the regulation of its bioavailability (34). Our study strongly supports the previous report that ANGPTL4 promotes capillary and/or lymph duct invasion as the first step of cancer metastasis.

The expression of ANGPTL4 is correlated with venous invasion, which may lead to distant metastasis to other organs through blood flow (15). In this study, the cause of death in 13 of the follow-up cases was distant metastasis to other organs, such as liver, lung or brain (Table IV). Further, all 13 cases with distant metastases were positive for ANGPTL4. We hypothesized worse survival outcome of the patients with ANGPTL4 due to the high incidence of distant metastasis. Although cancer patients with ANGPTL4 had relatively worse prognosis, there was no statistical difference in overall survival of patients with or without ANGPTL4 positivity. Prognostic investigation showed specifically that 4 of 9 cancer-related deaths showed ANGPTL4 negativity and local recurrence (Table IV). Some reports indicated that ANGPTL4 regulates cell motility and invasiveness (16,17). These findings suggest that the carcinoma with ANGPTL4 is more susceptible to distant metastasis and the carcinoma without ANGPTL4 shows a tendency for stromal invasion. The examination of ANGPTL4 in carcinoma tissue may predict the incidence of distant metastasis in human colorectal cancer. However, only 60 cases were investigated for follow-up in this study. Further investigation for detailed prognostic evaluation using substantially more cases is warranted.

ANGPTL4 was expressed in human colorectal adenocarcinoma and was correlated with several clinicopathological factors, especially venous invasion and distant metastasis. These findings suggest that ANGPTL4 is one of the factors involved in the progression of human colorectal cancer. However, the detailed mechanisms of ANGPTL4 protein in human colorectal cancer requires further investigation.

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