Expression of factors and key components associated with the PI3K signaling pathway in colon cancer

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Abstract. The pathophysiology of colorectal cancer (CRC) has not been fully elucidated. The dysregulation of the phosphatidylinositol 3-kinase (PI3K) signaling pathway frequently contributes to the tumorigenesis and progression of human cancer. The aim of the present study was to explore the expression and clinical significance of a number of associated factors and key components of the PI3K signaling pathway, including phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit α (p110α), phosphorylated protein kinase B (p-Akt) Ser473, p-mammalian target of rapamycin (mTOR) Ser2448, cyclin D1, cyclin dependent kinase (CDK)4, RELA proto-oncogene, nuclear factor-κβ subunit (p65), Ras and extracellular signal-regulated kinase (ERK)1/2 in human CRC. The expression of target proteins was detected using immunohistochemistry (IHC) in 65 CRC cases and 15 colonic adenoma cases. The association between the expression of target proteins and clinical pathological parameters was analyzed using a χ^2 test. IHC results revealed that the expression of all target proteins was significantly increased in CRC tissues compared with in colonic adenoma tissues ($P<0.05$). No significant associations were observed between the expression of p110α, p-Akt Ser473, p-mTOR Ser2448 and sex, age, differentiation, lymph node metastasis or Tumor-Node-Metastasis staging ($P>0.05$). Cyclin D1, CDK4 and Ras were revealed to be expressed significantly higher in poorly differentiated CRC compared with moderately differentiated CRC ($P<0.05$). Expression of p65 and ERK1/2 were significantly increased in cancer tissues with lymph node metastasis compared with cancer tissues without lymph node metastasis ($P<0.05$). These results suggest that the target proteins may all participate in the tumorigenesis of CRC. Furthermore, cyclin D1, CDK4, Ras, p65 and ERK1/2 may be important in the progression of CRC. The results of the present study may provide novel predictive factors and therapeutic targets for CRC.

Introduction

Colorectal cancer (CRC) is becoming one of the most common malignancies globally (1). Each year, almost 1.4 million new CRC cases occur and 700,000 individuals succumb to mortality from CRC globally (1-3). Despite advancements in surgery, radiation therapy, chemotherapy and targeted therapy, the survival rate of patients remains low (4,5). Although extensive studies have demonstrated that CRC is a multi-factorial disease and is associated with physical, chemical, biological and genetic factors (6), the pathophysiology of CRC remains to be fully elucidated.

The phosphatidylinositol 3-kinase (PI3K) signaling pathway serves crucial functions in normal cellular processes, including cell proliferation, growth, apoptosis and motility (7). PI3K, protein kinase B (Akt) and mammalian target of rapamycin (mTOR) represent key nodes in the PI3K pathway. Class IA PI3K is a heterodimeric lipid kinase composed of a catalytic subunit, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit α (p110α) and a regulatory subunit (p85) (8). Akt is the downstream effector of PI3K and regulates the effects of PI3K on tumor growth and progression (9,10). The phosphorylation at serine 473 in its C-terminus fully activates Akt, and the activity of Akt may be evaluated using antibodies against phosphorylated (p)-Akt-S473 (11,12). An evolutionarily conserved serine/threonine kinase known as mTOR is the downstream target of Akt signaling. Akt may directly phosphorylate serine 2448 in mTOR (9,13). Akt serves important functions in cell proliferation. Cyclin D1 is a key cell cycle regulatory protein and its expression levels are important in the G1/S phase transition (7,14). The activation of Akt may phosphorylate and inhibit glycogen synthesis kinase-3 β, which results in the stabilization of cyclin D1 (14). Activated Akt may

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control the assembly of cyclin D1-cyclin dependent kinase (CDK)4 complexes by modulating cyclin D1 levels and contributing to the regulation of cell cycle progression (15). Akt may also affect cell survival by the indirect regulation of the nuclear factor-κβ (NF-κβ) signaling pathway (7). However, the elevated expression of RELA proto-oncogene, NF-κβ subunit (p65), a subunit of activated NF-κβ, may increase the phosphorylation and expression of Akt in the NIH3T3 cell line and primary endothelial cells (16). The Ras oncoprotein is one of the main regulators of the NF-κβ signaling pathway, and in its active conformation, Ras may phosphorylate the Raf/mitogen-activated protein kinase kinase (MEK)/extracellular signal-regulated kinase (ERK) and the PI3K/Akt/mTOR signaling pathways to regulate normal cell growth and malignant transformation (17). Studies have demonstrated that the PI3K/Akt/mTOR and the Raf/MEK/ERK cascades are associated, with multiple points of convergence, cross-talk and feedback loops (10,17). ERK1/2 may mediate the phosphorylation of the essential scaffolding protein of mTOR complex 1 (C1) to promote mTOR signaling (18). The associated factors and key components of the P13K pathway include p110α, Akt, mTOR, cyclinD1, p65, Ras and ERK1/2; the aforementioned studies suggest that these factors may constitute a biochemical network to regulate cellular functions.

Numerous studies have demonstrated that the altered expression and/or activation of the aforementioned factors contribute to the tumorigenesis and progression of various types of human cancer, including CRC (7,10,13,14,17,19-31). However, systemic research on the expression and function of all these factors in CRC is limited.

In the present study, the expression of the associated factors and key components of the PI3K pathway in patients with CRC, including p110α, p-Akt Ser473, p-mTOR Ser2448, cyclin D1, CDK4, p65, Ras and ERK1/2, were explored. Their clinical significance in CRC was additionally analyzed.

Materials and methods

Patients and controls. Between January 2013 and December 2014, a total of 65 CRC and 15 colonic adenoma tissue samples were collected from the Department of Pathology of the Jinan Central Hospital Affiliated to Shandong University (Shandong, China) for a retrospective study. All tissues were confirmed by postoperative pathology. The group of colonic adenoma cases comprised 7 females and 8 males with an age range of 34-82 years (mean age, 64.2). The group of patients with CRC consisted of 34 males and 31 females, and the mean age was 66 years (range, 32-88 years). A total of 36 of the tumors were moderately differentiated and 29 cases were poorly differentiated according to pathology reports. According to the tumor-node-metastasis (TNM) staging system (32), there were 3 cases of stage I, 25 cases of stage II, 24 cases of stage III and 13 cases of stage IV. Lymph node metastasis occurred in 33 CRC cases. The present study was approved by the Institutional Review Board of Jinan Central Hospital Affiliated to Shandong University, and written informed consent was obtained from all patients or the patient's family.

Immunohistochemistry (IHC). IHC was performed to detect the expression of p110α, p-Akt Ser473, p-mTOR Ser2448, cyclin D1, CDK4, p65, Ras and ERK1/2 in CRC and colonic adenoma tissues. All tissue samples were 10% formalin-fixed at room temperature for 1 h, paraffin-embedded, cut into 4-μm sections and then placed on slides pretreated with 3-aminopropyltriethoxysilane. Following deparaffinization and rehydration (xylene for 10 min; 100, 95, 75 and 50% EtOH for 2 min each; PBS for 5 min), the sections were microwaved for heat-induced epitope retrieval at 100˚C for 3 min in a citrate buffer solution (10 mM sodium citrate and 0.05% Tween 20; pH 6.0). The sections were then washed using PBS and the endogenous peroxidase activity was inhibited with 3% hydrogen peroxide for 10 min. The specimens were then blocked with PBS containing 5% normal goat serum (cat no., ab7481; Abcam, Shanghai, China) at 37˚C for 30 min. Subsequently, the sections were incubated with antibodies overnight at 4˚C. The primary antibodies used included a rabbit anti-p110α antibody (cat no. 4249; 1:200), a rabbit anti-p-Akt Ser473 antibody (cat no. 4060; 1:200), a rabbit anti-p-mTOR Ser2448 antibody (cat no. 2976; 1:200), a rabbit anti-cyclin D1 antibody (cat no. 2978; 1:200), a rabbit anti-CDK4 antibody (cat no. 12790; 1:200), a rabbit anti-NF-κB p65 antibody (cat no. 8242; 1:200), a rabbit anti-Erk1/2 antibody (cat no. 4695; 1:200; all Cell Signaling Technology, Inc., Danvers, MA, USA) and a rabbit anti-Ras (H,K,N) antibody (cat no. LS-C176193; 1:200; LifeSpan BioSciences, Inc., Seattle, WA, USA). Following rinsing with PBS, the slides were incubated at room temperature for 30 min with a secondary anti-rabbit antibody conjugated with horseradish peroxidase (1:200; cat no., sc-2357; Santa Cruz Biotechnology, Inc., Dallas, TX, USA). The slides were then exposed to 3,3'-diaminobenzidine for visualization, and hematoxylin at room temperature for 5 min, for nuclear counterstaining.

Evaluation of immunostaining parameters. Tissue sections were evaluated at a x200 magnification using light microscopy by two pathologists in a blinded manner. Slides with debatable evaluations were re-evaluated until a consensus was reached. First, proportion scores were assigned to represent the estimated proportion of positive tumor cells (0, <5%; 1, 6-25%; 2, 26-50%; 3, 51-75%; 4, ≥76%). Secondly, intensity scores were assigned to represent the average intensity of the positive tumor cells (0, no color; 1, pale yellow; 2, tan; 3, brown). The proportion and intensity scores were then multiplied to obtain total scores. A total of 5 typical regions were assessed for each sample and the average scores were obtained. For further statistical analysis, all specimens were divided into four groups: Negative expression (-), 0-1; weak positive expression (+), 2-4; positive expression (++), 5-8; or strong positive expression (+++) >9. Scores with a range from 1-2 were also added to the positive expression group.

Statistical analysis. SPSS 17.0 (SPSS, Inc., Chicago, IL, USA) software package was used for statistical analyses. The χ^2 test was performed to analyze the differences in various target proteins between CRC tissues and colonic adenoma tissues, in addition to associations between the expression levels of the various proteins and the clinical parameters. $P<0.05$ was considered to indicate a statistically significant difference.

Table I. Expression of different targets in colon cancer and colonic adenoma tissues.

Groups	-	+	++	+++	Positive number	Positive rate (%)	P-value
p110α							<0.001
Colon cancer	13	22	22	8	52	80.00	
Colonic adenoma	12	2	1	0	3	20.00	
p-Akt Ser473							0.001
Colon cancer	17	30	14	4	48	73.85	
Colonic adenoma	10	5	0	0	5	33.33	
Cyclin D1							<0.001
Colon cancer	20	26	13	6	45	69.23	
Colonic adenoma	13	2	0	0	2	13.33	
CDK4							0.005
Colon cancer	24	26	11	4	41	63.08	
Colonic adenoma	12	2	1	0	3	20.00	
p-mTOR Ser2448							0.002
Colon cancer	14	22	16	13	51	78.46	
Colonic adenoma	11	1	2	1	4	26.67	
NF-κβ							0.021
Colon cancer	22	17	17	9	43	66.15	
Colonic adenoma	10	3	1	1	5	33.33	
RAS							
Colon cancer	25	16	15	9	40	61.54	0.022
Colonic adenoma	11	2	1	1	4	26.67	
ERK1/2							0.019
Colon cancer	19	25	19	2	46	70.77	
Colonic adenoma	11	1	2	1	4	26.67	

CDK, cyclin dependent kinase; ERK, extracellular signal-regulated kinase; p65, RELA proto-oncogene, nuclear factor-Kβ subunit; p-, phosphorylated; Akt, protein kinase B; mTOR, mammalian target of rapamycin; p110α, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit α.

Results

Expression of p110α, p-Akt Ser473, cyclin D1, CDK4, p-mTOR Ser2448, p65, Ras and ERK1/2 in CRC tissues and colonic adenoma tissues. IHC staining results revealed that, of the 65 CRC cases, 52 cases (80.00%) were p110α-positive, 48 cases (73.85%) were p-Akt Ser473-positive, 45 cases (69.23%) were cyclin D1-positive, 41 cases (63.08%) were CDK4-positive, 51 cases (78.46%) were p-mTOR Ser2448-positive, 43 cases (66.15%) were p65-positive, 40 cases (61.54%) were Ras-positive, and 46 cases (70.77%) were ERK1/2-positive. However, positive staining of p110α, p-Akt Ser473, cyclin D1, CDK4, p-mTOR Ser2448, p65, Ras and ERK1/2 was only observed in 3 cases (20.00%), 5 cases (33.33%), 2 cases (13.33%), 3 cases (20.00%), 4 cases (26.67%), 5 cases (33.33%), 4 cases (26.67%) and 4 cases (26.67%) of 15 colon adenoma cases, respectively. The expression of target proteins in CRC tissues were all significantly increased compared with in colon adenoma tissues (Table I; P<0.05). The localization of p110α and p-Akt Ser473 were primarily in the cytoplasm of CRC cells (Fig. 1A and B). The location of p-mTOR Ser2448 was mainly in the perinuclear and cytoplasmic regions of CRC cells, and in the nuclei of colonic adenoma cells (Fig. 1C). Cyclin D1 and CDK4 presented in the nuclei of CRC cells (Fig. 2A and B). The localization of p65 was mainly in the nuclei and cytoplasm of CRC cells (Fig. 2C). Ras and ERK1/2 were also observed in the cytoplasm of CRC cells (Fig. 3A and B).

Association between target proteins and clinicopathological characteristics in CRC. Statistical analysis results indicated that p110α, p-Akt Ser473 and p-mTOR Ser2448 expression were not significantly associated with sex, age, degree of differentiation, lymphatic metastasis or TNM staging of patients with CRC (P>0.05; Table II). No significant association was identified between cyclin D1 or CDK4 expression and sex, age and lymphatic metastasis (P>0.05; Table III). The positive expression of cyclin D1 and CDK4 in stage I and stage II of CRC were significantly increased compared with in stage III and stage IV (P<0.05; Table III). Tumors in poorly differentiated CRC presented a significantly increased positive expression of cyclin D1 or CDK4 compared with tumors in moderately differentiated CRC (P<0.05; Table III).

Table II. Association between the expression of p110α, p-Akt Ser473 and p-mTOR Ser2448 and clinicopathological characteristics.

		p110α					p-Akt Ser473					p-mTOR Ser2448				
Parameters	n	-	+	++	+++	P-value	-	+	++	+++	P-value	-	+	++	+++	P-value
Sex						0.081					0.628					0.445
Male	34	8	15	7	4		9	17	6	2		8	11	11	4	
Female	31	5	7	15	4		8	13	8	2		6	11	5	9	
Age, years						0.831					0.988					0.494
≥60	41	7	16	14	4		11	19	7	4		10	14	9	8	
<60	24	6	6	8	4		6	11	7	0		4	8	7	5	
Degree of differentiation						0.494					0.385					0.191
Moderate	36	7	10	15	4		9	15	9	3		7	10	10	9	
Poor	29	6	12	7	4		8	15	5	1		7	12	6	4	
Lymphatic metastasis						0.07					0.498					0.728
Yes	33	5	8	16	4		12	15	8	4		5	15	5	8	
No	32	8	14	6	4		9	15	6	0		9	7	11	5	
TNM staging						0.053					0.102					0.263
I+II	28	7	13	5	3		9	14	5	0		9	6	10	3	
III+IV	37	6	9	17	5		8	16	9	4		5	16	6	10	

p-, phosphorylated; Akt, protein kinase B; mTOR, mammalian target of rapamycin; TNM, tumor-node-metastasis; p110α, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit α.

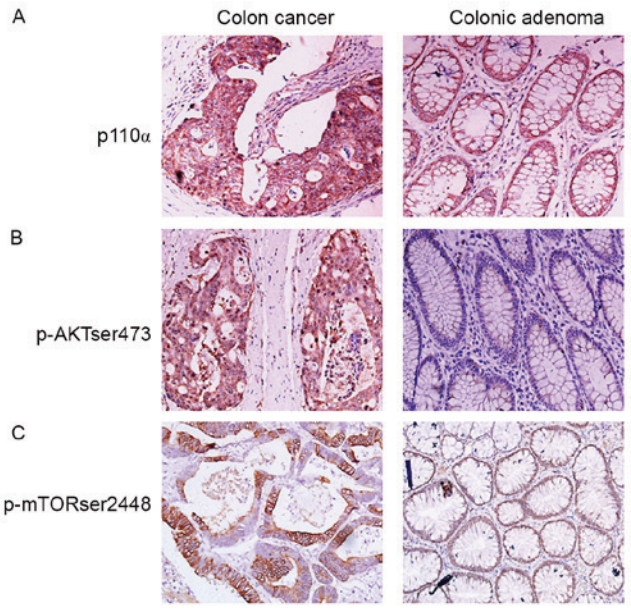

Figure 1. Immunohistochemical staining of p110α, p-Akt Ser473 and p-mTOR Ser2448 in CRC tissues and colonic adenoma tissues. Staining of (A) p110α, (B) p-Akt Ser473 and (C) p-mTOR Ser2448 in CRC and colonic adenoma tissue (magnification, x200). CRC, colorectal cancer; p110α, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit α; p-, phosphorylated; Akt, protein kinase B; mTOR, mammalian target of rapamycin.

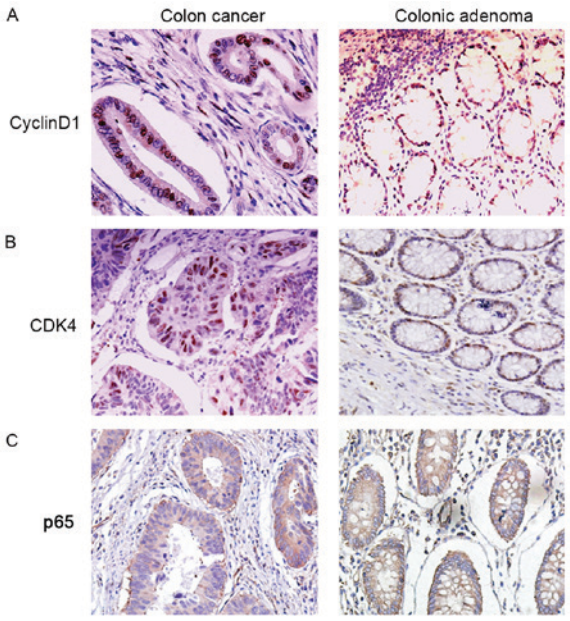

Figure 2. Immunohistochemical staining of cyclin D1, CDK4 and p65 in CRC tissues and colonic adenoma tissues. Staining of (A) cyclin D1, (B) CDK4 and (C) p65 in CRC and colonic adenoma tissue (magnification, x200). CRC, colorectal cancer; CDK, cyclin dependent kinase; p65, RELA proto-oncogene, nuclear factor-κβ subunit.

The expression of p65 was significantly increased in cancer tissues with lymph node metastasis compared with in cancer tissues without lymph node metastasis (P<0.05; Table III). The expression of p65 significantly decreased in stage I and stage II of CRC compared with stage III and stage IV of CRC (P<0.05; Table III). However, the expression of p65 was not associated with sex, age and degree of differentiation (P>0.05; Table III). The positive expression of Ras was significantly increased in poorly differentiated CRC compared with moderately differentiated CRC (P<0.05;

Table III. Association between the expression of cyclin D1, CDK4 and p65 and clinicopathological characteristics.

		Cyclin D1					CDK4					p65				
Parameter	n	-	+	++	+++	P-value	-	+	++	+++	P-value	-	+	++	+++	P-value
Sex						0.48					0.823					0.163
Male	34	11	14	8	1		12	14	6	2		14	9	7	4	
Female	31	9	12	5	5		12	12	5	2		8	8	10	5	
Age, years						0.487					0.701					0.329
≥60	41	14	15	10	2		16	16	6	3		14	14	8	5	
<60	24	6	11	3	4		8	10	5	1		8	3	9	4	
Degree of differentiation						0.01					0.008					0.69
Moderate	36	16	12	7	1		18	13	4	1		14	8	8	6	
Poor	29	4	14	6	5		6	13	7	3		8	9	9	3	
Lymphatic metastasis						0.562					0.067					0.008
Yes	33	7	12	9	5		9	14	7	3		6	11	8	8	
No	32	13	14	4	1		15	12	4	1		16	6	9	1	
TNM staging						0.002					0.034					0.006
I+II	28	13	12	2	1		14	10	4	0		15	5	7	1	
III+IV	37	7	14	11	5		10	16	7	4		7	12	10	8	

CDK, cyclin dependent kinase; p65, RELA proto-oncogene, nuclear factor-κβ subunit; TNM, tumor-node-metastasis.

Table IV. Association between the expression of Ras and ERK1/2 and clinicopathological characteristics.

		Ras					ERK1/2				
Parameter	n	-	+	++	+++	P-value	-	+	++	+++	P-value
Sex						0.962					0.527
Male	34	13	9	7	5		13	9	11	1	
Female	31	12	7	8	4		6	16	8	1	
Age, years						0.820					0.977
≥60	41	16	8	12	5		12	16	11	2	
<60	24	9	8	3	4		7	9	8	0	
Degree of differentiation						0.002					0.308
Moderate	36	17	6	9	4		9	14	11	2	
Poor	29	8	10	6	5		10	11	8	0	
Lymphatic metastasis						0.426					0.033
Yes	33	11	12	8	1		6	13	13	1	
No	32	14	4	7	8		13	12	6	1	
TNM staging						0.260					0.036
I+II	28	12	3	6	8		13	8	6	1	
III+IV	37	13	13	9	1		6	17	13	1	

ERK, extracellular signal-regulated kinase; TNM, tumor-node-metastasis.

Table IV). However, the expression of RAS was not associated with sex, age, TNM staging or lymphatic metastasis (P>0.05; Table IV). The expression of ERK1/2 was significantly increased in lymphatic metastasized tissues compared with non-lymphatic metastasized tissues (P<0.05; Table IV). The expression of ERK1/2 significantly decreased in stage I and stage II of CRC compared with stage III and stage IV of CRC (P<0.05; Table IV). There was no significant association indicated between the expression of ERK1/2 and sex, age or degree of differentiation (P>0.05; Table IV).

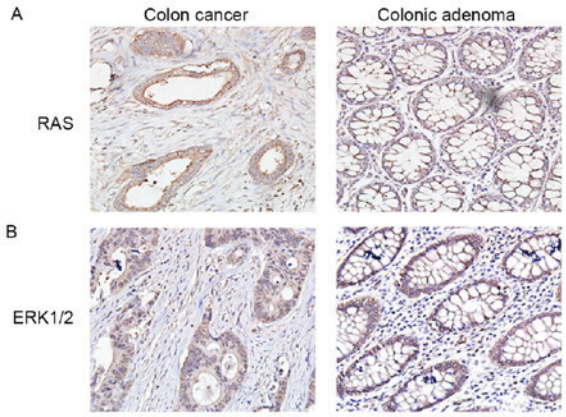

Figure 3. Immunohistochemical staining of Ras and ERK1/2 in CRC tissues and colonic adenoma tissues. Staining of (A) Ras and (B) ERK1/2 in CRC and colonic adenoma tissue (magnification, x200). CRC, colorectal cancer; ERK, extracellular signal-regulated kinase.

Discussion

In the present study, the expression of the associated factors and key components of the PI3K signaling pathway in CRC tissues and colonic adenoma tissues were evaluated, and their clinical significances analyzed. These components include p110α, p-Akt Ser473, p-mTOR Ser2448, cyclin D1, CDK4, p65, Ras and ERK1/2. The expression of the target proteins was all significantly increased in CRC tissues compared with in colonic adenoma tissues (P<0.05). The expression of cyclin D1, CDK4 and Ras were significantly increased in poorly differentiated CRC compared with moderately differentiated CRC (P<0.05). The expression of p65 and ERK1/2 were significantly increased in cancer tissues with lymph node metastasis compared with in cancer tissues without lymph node metastasis (P<0.05).

p110α protein is the catalytic subunit of PI3K. The amplification of the genes encoding p110α have been demonstrated in ovarian, breast and pancreatic cancer cells (14). A previous study demonstrated that clustered regions of point mutations in the p110α catalytic subunit occurred in 20-30% of the breast, colon, brain and gastric tumors examined (10). Additionally, the overexpression of the regulatory subunit of PI3K (p85) protein and mutations in the gene encoding p85 have been identified in CRC (13,21). The aforementioned previous studies provide direct evidence for the alteration of PI3K in human cancer types. In the present study, it was revealed that the expression of p110α is significantly increased in CRC tissues compared with in colonic adenoma tissues (P<0.05; Table I). However, its expression was not associated with the sex, age, degree of differentiation, lymphatic metastasis or TNM staging of patients with CRC (P>0.05; Table II). The results of the present study suggest that p110α may be involved in the tumorigenesis of CRC.

Akt is the downstream effector of PI3K and regulates the effects of PI3K on tumor growth and progression (9,10). The complete activation of Akt requires phosphorylation at Serine 473 in its C-terminus (11,12). Akt phosphorylation has been observed in patients with non-small cell lung cancer (22,23), and Akt phosphorylation in human CRC was associated with the clinicopathological parameters of patients (24,25). In the present study, it was revealed that the expression of p-Akt Ser473 was significantly increased in CRC tissues compared with in colonic adenoma tissues (P<0.05; Table I). However, its expression was not associated with the sex, age, degree of differentiation, lymphatic metastasis or TNM staging of patients with CRC (P>0.05; Table II). The results of the present study suggest that p-Akt Ser473 may be involved in the tumorigenesis of CRC.

mTOR is the downstream target of Akt signaling and Akt may directly phosphorylate its Serine 2448 (10). The abnormal expression of p-mTOR Ser2448 has been linked to tumor progression in a number of types of human cancer, including CRC (13,26). In the present study, it was revealed that the expression of p-mTOR Ser2448 was significantly increased in CRC tissues compared with in colonic adenoma tissues (P<0.05; Table I). However, its expression was not associated with the sex, age, degree of differentiation, lymphatic metastasis or TNM staging of patients with CRC (P>0.05; Table II). The results of the present study suggest that p-mTOR Ser2448 may be involved in the tumorigenesis of CRC.

Cyclin D1 is a key cell cycle regulatory protein, and may form a complex with CDK4 in order to govern the cell cycle and its progression (7,14). Akt may indirectly regulate the levels of cyclin D1 and the assembly of cyclin D1-CDK4 complexes (14,15). The altered expression of cyclin D1 and CDK4 has been demonstrated in a variety of tumor types, including colon tumors (27-29). They may be involved in early events in gastric tumorigenesis (29). In the present study, it was revealed that the expression levels of cyclin D1 and CDK4 were significantly increased in CRC tissues compared with those in colonic adenoma tissues (P<0.05; Table I). The expression levels of cyclin D1 and CDK4 were significantly increased in poorly differentiated CRC compared with moderately differentiated CRC (P<0.05). The results of the present study suggest that cyclin D1 and CDK4 may participate in the tumorigenesis and progression of CRC.

The NF-κβ signaling pathway serves important functions in oncogenesis by regulating the expression of genes associated with the development and progression of cancer (20). Akt may affect cell survival by indirectly regulating the NF-κβ signaling pathway (7). p65, the subunit of activated NF-κβ, has been demonstrated to regulate the phosphorylation and expression of Akt (16). An abnormal expression of p65 was observed in pancreatic cancer, in which a high expression of p65 indicated poor patient survival (30). In the present study, it was revealed that the expression of p65 was significantly increased in CRC tissues compared with that in colonic adenoma tissues (P<0.05; Table I). The expression of p65 was significantly increased in cancer tissues with lymph node metastasis compared with in cancer tissues without lymph node metastasis (P<0.05; Table III). These results suggest that p65 may be associated with the tumorigenesis and progression of CRC.

Ras proteins may regulate the phosphorylation of the Raf/MEK/ERK and the PI3K/Akt/mTOR signaling pathways (17). They exhibit essential functions in controlling a number of the malignant characteristics of transformed cells, for example in breast cancer (17,18). The activated mutations in the Ras genes themselves or alterations in upstream or downstream signaling components occurred in various types of

human cancer, including CRC (10,33). KRAS proto-oncogene, GTPase (KRAS) is a member of the Ras family, and its mutations are predictors for resistance to cetuximab therapy in CRC (34). KRAS expression was significantly increased in carcinomatous lesions of the pancreas compared with in normal ducts or ductal hyperplasia (35). The antibody used in the present study may detect the expression of the three members of the Ras family (namely, HRAS proto-oncogene, GTPase, KRAS and NRAS proto-oncogene, GTPase). It was revealed that the expression of Ras was significantly increased in CRC tissues compared with in colonic adenoma tissues ($P<0.05$; Table I). The positive expression rate of Ras was significantly increased in poorly differentiated CRC compared with that in moderately differentiated CRC ($P<0.05$; Table IV). The results of the present study suggest that Ras may participate in the tumorigenesis and progression of CRC.

ERK1 and ERK2 are MAP kinases and serve important functions in normal physiological development and carcinogenesis (17,36). ERK1/2 may mediate the phosphorylation of the essential scaffolding protein of mTORC1 in order to promote mTOR signaling (18). ERK1 expression may be an early marker of cervical carcinogenesis (37). In the present study, it was revealed that the expression of ERK1/2 was significantly increased in CRC tissues compared with in colonic adenoma tissues ($P<0.05$; Table I). The expression of ERK1/2 was significantly increased in lymphatic metastasized tissues compared with non-lymphatic metastasized tissues ($P<0.05$; Table IV). The results of the present study suggest that ERK1/2 may participate in the tumorigenesis and progression of CRC.

In conclusion, the results of the present study demonstrated that p110α, p-Akt Ser473, p-mTOR Ser2448, cyclin D1, CDK4, p65, Ras and ERK1/2 may all participate in the tumorigenesis of CRC. Additionally, cyclin D1, CDK4, Ras, p65 and ERK1/2 may be important in the progress of CRC. These results may provide novel predictive factors and therapeutic targets for CRC.

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