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Original Article

Expression of PI3Kp110α and PI3Kp110β in the colorectal conventional adenoma, serrated lesions and adenoma with canceration and their significance

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Abstract: Aims: To evaluate the expression and clinical significance of PI3Kp110α and PI3Kp110β in colorectal conventional adenoma, serrated lesions and adenoma with canceration. Methods and results: Immunohistochemistry and Western blot analysis were conducted to detect the expression of p110α and p110β in normal colorectal tissues, conventional adenoma, serrated lesions and adenoma canceration. Results revealed that the expression of P110α and P110β in the adenoma canceration was significantly higher than that in normal tissues, tubular adenoma (low grade) and tubular-villous adenoma (low grade) of conventional adenoma, hyperplastic polyps of serrated lesions ($P<0.05$). But there was no significant difference between the adenoma canceration and the high grade adenoma of conventional adenoma, all grade of villous adenoma and serrated adenoma ($P>0.05$). The expression of p110α and p110β was correlated with different clinicopathologic factors in conventional adenoma, serrated adenoma and adenoma canceration ($P<0.05$). Conclusions: p110α and p110β were highly expressed in villous adenoma, serrated adenoma and adenoma with canceration. Its high expression may be the risk factor of the progress of adenoma to adenocarcinoma, and may be an important cause of what canceration rate of villous adenoma and serrated adenoma was higher than that of other adenomas. Combined detection of p110α and p110β is helpful to determine the canceration potential of colorectal villous adenoma and serrated adenoma.

Keywords: Colorectal adenoma, adenocarcinoma, PI3Kp110α, PI3Kp110β

Introduction

Colorectal adenoma is a common disease of the digestive system and accounts for approximately 95% of colorectal cancer that originated from colorectal adenoma [1]. Colorectal adenoma is divided into conventional adenoma and serrated lesions [2]. Conventional adenomas include tubular adenoma (TA), tubular-villous adenoma (TVA) and villous adenoma (VA). Serrated lesions include hyperplastic polyp (HP), sessile serrated adenoma (SSA) and traditional serrated adenoma (TSA). The conventional adenoma-carcinoma sequence has been widely recognized. And in recent years, many scholars have established the serrated neoplasia pathway, which starts from HP proliferation to serrated adenoma (SA) and then malignant adenocarcinoma. About 60% of colorectal cancer started from conventional adenoma and 35% from the serrated pathway [3]. Therefore, it is of great significance to study the risk factor and the molecular mechanism of the malignant transformation of the sequences. In the present study, we evaluated the expression of p110α and p110β which are the core molecules of the phosphatidylinositol 3-kinase/AKT (PI3K/AKT) signaling pathway in normal colorectal tissues, conventional adenomas, serrated lesions and adenoma cancerations, and aims to explore the expression and clinical significance of these PI3Kp110 subunits in the conventional adenoma-carcinoma sequence and the serrated neoplasia pathway.

Materials and methods

Clinical specimens and patient data

A total of 520 colorectal tissue samples at different stages were collected from the Department of Pathology of Binzhou Medical University Hospital and Binzhou People's Hospital between January 2009 and August 2014. Samples

included conventional adenomas (TA, TVA and VA), serrated lesions (HP, SSA and TSA), respectively, 60; adenocarcinoma (tubular adenoma cancerations, villous adenoma cancerations and serrated adenoma cancerations) and normal tissues, respectively, 40. Parts of the tissue samples were immediately frozen in liquid nitrogen, and parts were routinely fixed in 10% buffered formalin liquid. Tumor tissues were cut into wedge shapes, and normal tissues were cut at least 5 cm away from the tumor margin. Adenoma canceration tissues were the coexistence of invasive carcinoma and adenoma. Demographic and clinicopathological parameters were prospectively recorded using a chart review.

All patients were clinically and pathologically proven to have not received preoperative chemotherapy or radiotherapy. Prior to specimen collection, the patients provided their informed consents and the Ethical Committee of Binzhou Medical University Hospital approved the protocols used. All procedures performed in studies involving human participants were in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Immunohistochemistry and scoring

Prior to staining, paraffin-embedded tissue blocks were cut into 4 μm thickness. The sections were deparaffinized in an oven at 60°C for 1 h and rehydrated with two and three changes of xylene and ethanol, respectively. Antigen retrieval was performed using the high-pressure hot retrieval method. Endogenous peroxidase activity was determined through incubation with 3% hydrogen peroxide for 10 min at room temperature. Nonspecific binding was blocked by incubating the sections with 10% normal goat serum in PBS for 30 min at room temperature. Without washing, the sections were incubated with rabbit monoclonal antibody against human PI3Kp110α (1:80; Abcam, Cambridge, MA, USA) and PI3Kp110β (1:50; Abcam, Cambridge, MA, USA) at 4°C overnight. The sections were then incubated with horseradish peroxidase-conjugated secondary goat anti-rabbit antibody (Abcam, San Francisco, USA) for 1 h at room temperature. The sections were then washed with PBS and treated with metal-enhanced DAB substrate kit (Thermo Scientific, USA) to visualize the antigen-antibody complex. Two researchers, who were unaware of the clinicopathological status of the specimens, scored each section separately. The percentage of stained cells on each section was scored as 0 (less than 5%), 1 (5% to 25%), 2 (26% to 50%), and 3 (>50%). Staining intensity was scored as 0 (no staining), 1 (weak staining), 2 (moderate staining), and 3 (strong staining). The final score of each specimen was calculated by multiplying the stained cell score with the staining intensity score. The final score ranged from 0 to 9. Low expression was defined as a final score of <4, whereas a score ≥4 was considered as high expression.

Western blot analysis

Tissue samples were homogenized in SDS buffer containing the protease inhibitor PMSF. The homogenates were incubated on ice for 20 min and then centrifuged at 12,000 rpm for 30 min at 4°C. Supernatant was collected and added with a similar volume of 2× SDS buffer. The mixture was boiled for 10 min and preserved at -20°C. The protein extracts (50 μg) were separated through SDS-PAGE and then transferred onto polyvinylidene difluoride membrane (Millipore, USA). The membranes were blocked with 5% nonfat milk in Tris-buffered saline containing 0.1% Tween-20 at room temperature for 90 min. The membranes were then immunoblotted for PI3Kp110α (1:1200; Abcam, Cambridge, MA, USA), PI3Kp110β (1:1500; Abcam, Cambridge, MA, USA) and β-actin (1:1000; Abcam, Cambridge, MA, USA). Proteins bands were detected with secondary antibodies conjugated to horseradish peroxidase and visualized with enhanced chemiluminescence reagents.

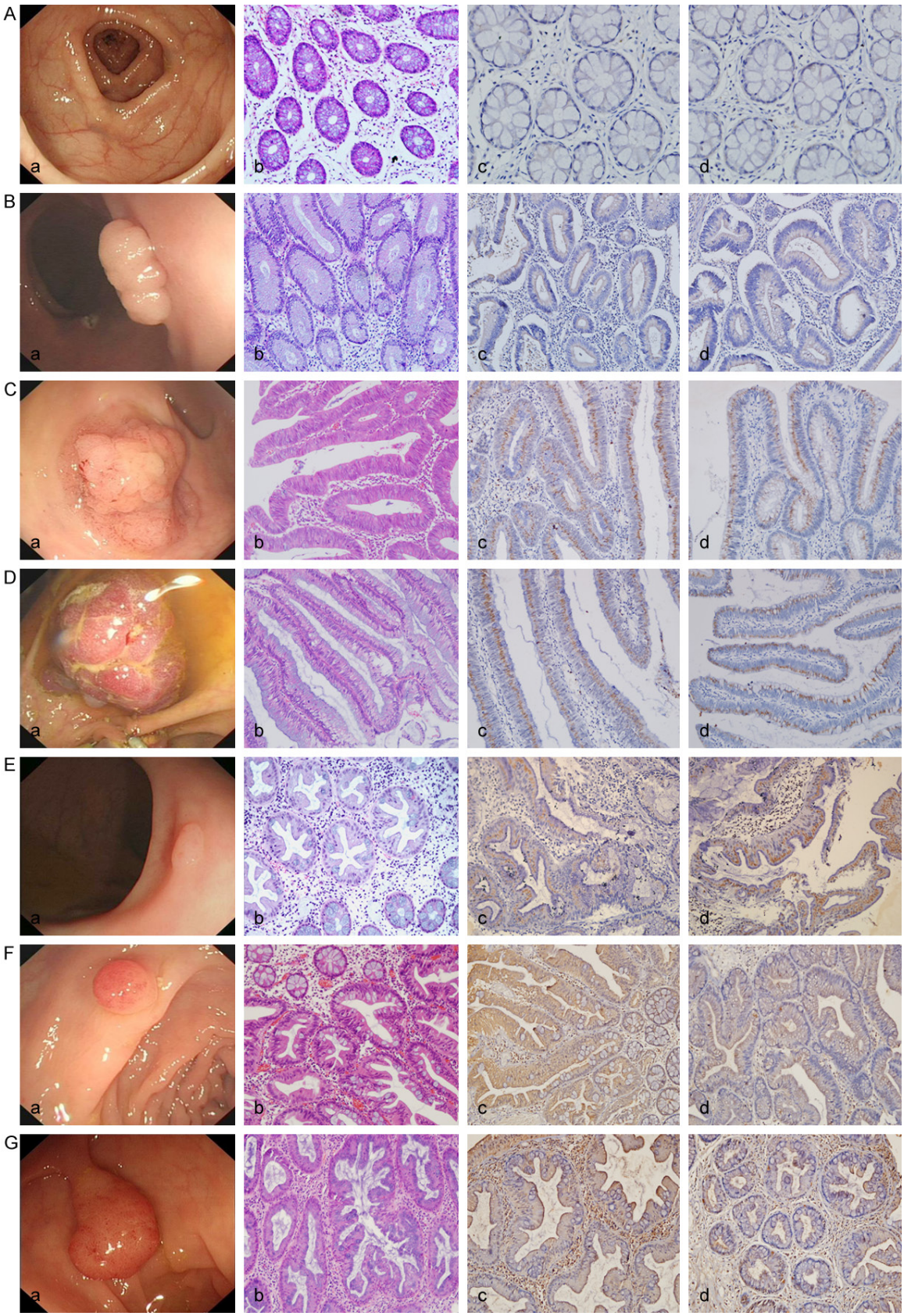
Statistical analysis

All statistical analyses were conducted with the SPSS 20.0 software (SPSS Inc., Chicago, USA). Differences between high and low expression were compared through X and Fisher's exact tests. Multiple linear regression analysis was used to analyze the relationship between p110α, p110β and the factors of clinical pathology. P value ≤0.05 was considered statistically significant.

Results

Expression and correlation of PI3Kp110α and PI3Kp110β in different diseased tissues by Immunohistochemistry

PI3Kp110α and PI3Kp110β showed different degrees of positive expression in normal mucosa, conventional adenoma, serrated lesions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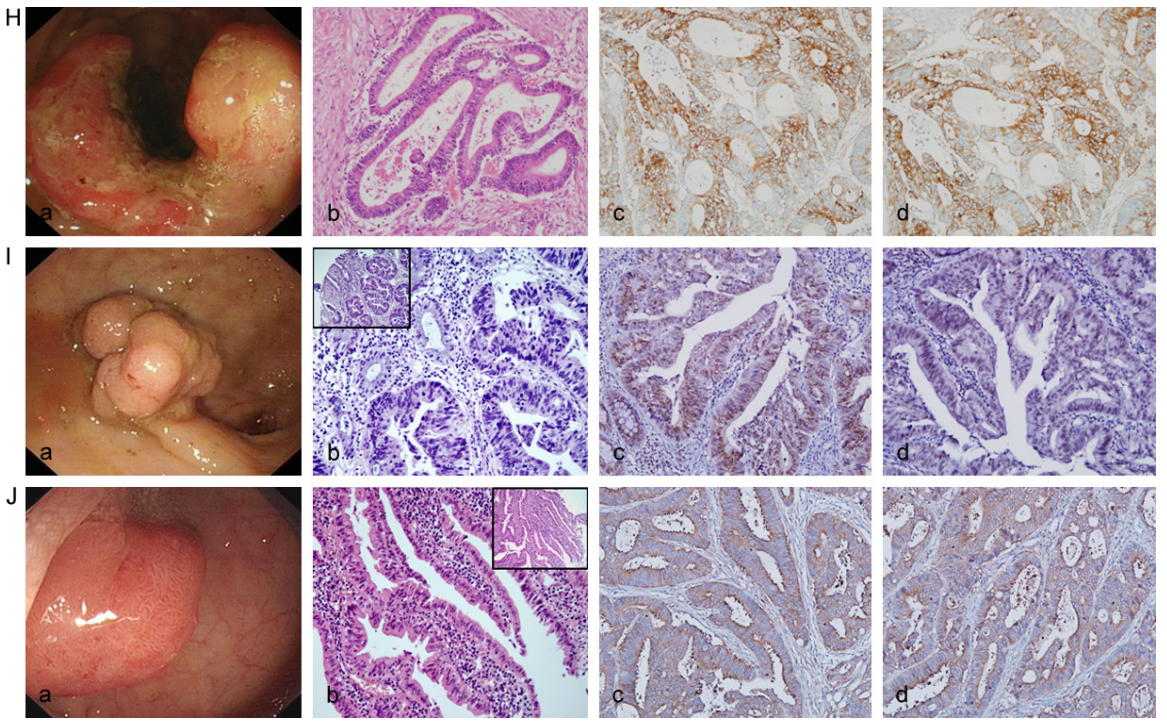

Figure 1. A: Colorectal normal tissue; B: TA; C. TVA; D: VA; E. HP; F: SSA; G: TSA; H: tubular adenocarcinoma; I: serrated adenoma canceration; J. villous adenoma canceration. a: Endoscopic; b: H&E (Original magnification × 200); c: Immunohistochemical PI3Kp110α staining (× 200); d: Immunohistochemical PI3Kp110β staining (× 200).

Table 1. PI3Kp110α and PI3Kp110β expression in different diseased colorectal tissues *n* (%)

Groups			N	p110α	p110β
Normal tissue			40	1 (2.5)	2 (5.0)
Conventional adenoma	TA	Low grade	31	8 (25.8)	7 (22.6)
		High grade	29	20 (69.0)	19 (65.5)
	TVA	Low grade	32	15 (46.9)	16 (50.0)
		High grade	28	20 (71.4)	21 (75.0)
	VA	Low grade	33	22 (66.7)	22 (66.7)
		High grade	27	24 (88.9)	22 (81.5)
Serrated lesions	HP		60	23 (38.3)	21 (35.0)
	SSA	Low grade	29	21 (72.4)	22 (75.9)
		High grade	31	24 (77.4)	25 (80.6)
	TSA	Low grade	30	23 (76.7)	23 (76.7)
		High grade	30	24 (80.0)	24 (80.0)
Adenocarcinoma	Tubular adenoma canceration		40	32 (80.0)	30 (72.5)
	Villous adenoma canceration		40	31 (77.5)	32 (80.0)
	Serrated adenoma canceration		40	32 (80.0)	33 (82.5)

and adenocarcinoma (**Figure 1**; **Table 1**). As shown by multiple paired χ^2 test of the binary data of the groups, expression levels of p110α and p110β in the neoplastic groups are higher than the normal mucosa group, and the difference is statistically significant ($P<0.05$).

Based on our analysis, we found that the expression of P110α and P110β was increased with the cancerous process of transforming from the normal mucosa into low grade adenoma, high grade adenoma, and finally into cancer in conventional adenoma-carcinoma sequence. There was no significant difference between the high-grade adenoma and adenoma canceration in TA and TVA. Similarly, there was no difference between all grade (low and high grade) adenoma and adenoma canceration in VA ($P>0.05$). However, there were significant differences among the other groups ($P<0.05$). In serrated neoplasia pathway, the expression of P110α and P110β had no significant difference between all grade SA and adenoma canceration ($P>0.05$), but both were significantly higher than that of HP ($P<0.05$).

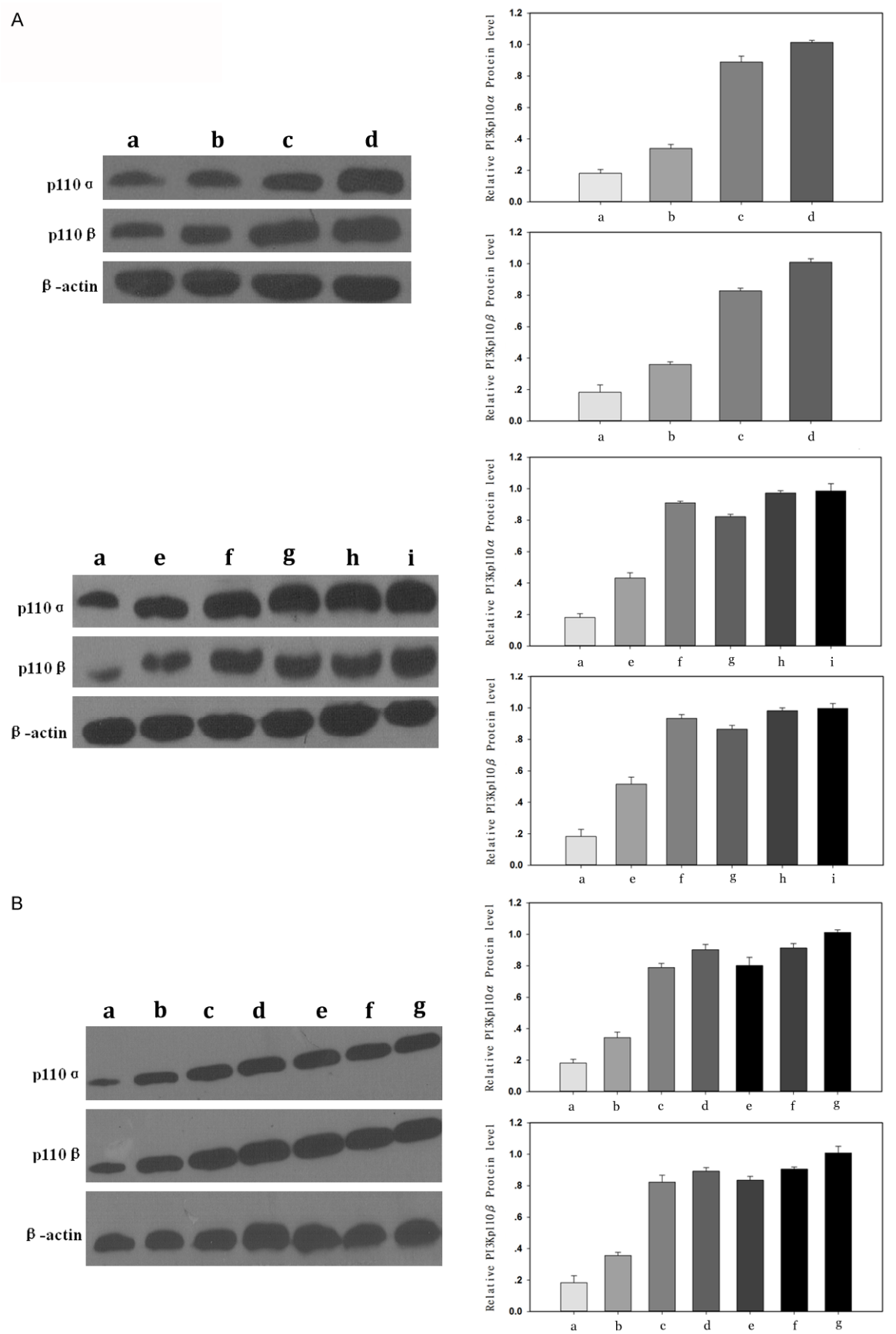
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Figure 2. Western blot analysis: A: PI3Kp110α and PI3Kp110β protein expression in conventional adenoma-carcinoma sequence. a: normal tissue; b: low grade of TA; c: high grade of TA; d: tubular adenocarcinoma; e: low grade of TVA; f: high grade of TVA; g: low grade of VA; h: high grade of VA; i: villous adenoma canceration. B: PI3Kp110α and PI3Kp110β protein expression in serrated neoplasia pathway. a: normal tissue; b: HP; c: low grade of SSA; d: high grade of SSA; e: low grade of TSA; f: high grade of TSA; g: serrated adenoma canceration.

Table 2. Correlation between p110α and p110β with different clinicopathologic parameters in colorectal conventional adenoma

Parameters	Tubular-villous adenoma					Villous adenoma				
	N	p110α (+)	*P* value	p110β (+)	*P* value	*N*	p110α (+)	*P* value	p110β (+)	*P* value
Gender										
Male	36	20	0.396	20	0.1179	37	28	0.818	28	0.603
Female	24	15		17		23	18		16	
Age (years)										
<60	35	21	0.484	22	0.516	20	13	0.131	15	0.836
≥60	25	16		15		40	33		29	
Tumor site										
Colon	42	25	0.497	26	0.588	40	33	0.131	31	0.302
Rectal	18	10		11		20	13		13	
Sizes (cm)										
<0.2	25	16	0.484	14	0.310	22	13	0.014※	11	0.002※
≥0.2	35	21		23		38	33		33	
Grade										
Low grade	32	15	0.048※	16	0.042※	33	22	0.041※	22	0.159
High grade	28	20		21		27	24		22	
Number										
Single	22	17	0.024※	12	0.063	46	37	0.211	32	0.232
Multiple	38	18		25		14	9		12	
Pedicle										
Yes	23	15	0.281	14	0.567	13	3	0.000※	4	0.000※
No	37	20		23		47	43		40	

※Indicate statistical significant (*P*<0.05).

Comparison between the two carcinogenesis pathways exhibited that p110α and p110β expression in the TA and TVA was lower than the low and high grade of SA, and the difference is statistically significant (*P*<0.05). But pairwise comparisons of the high grade subgroup, the VA group and SA group indicate that all the differences among these groups are not significant (*P*>0.05).

Expression of PI3Kp110α and PI3Kp110β in different diseased tissues by Western blot

The average relative expression levels of the PI3Kp110α and PI3Kp110β proteins in normal mucosa, conventional adenoma, serrated lesions and adenocarcinoma were different. Based on statistical analysis, the expression of P110α and P110β had no significant difference between high grade of TA and TVA, all grade of VA and SA and adenoma canceration (*P*>0.05). However, the expression was significantly higher than the low grade of TA and TVA, HP and normal mucosa (**Figure 2**).

Correlation of PI3Kp110α and PI3Kp110β with different clinicopathologic parameters

In conventional adenomas, p110α and p110β expression was correlated with the size, grade and number of TVA (*P*<0.05), and the size and with or without pedicle of VA (*P*<0.05) (**Table 2**). The expression was not correlated with the clinical pathological factors of TA (*P>0.05*). In serrated lesions, p110α and p110β expression was correlated with the size of SSA and TSA

Table 3. Correlation between p110α and p110β with different clinicopathologic parameters in colorectal serrated adenoma

Parameters	Sessile serrated adenoma					Traditional serrated adenoma				
	N	p110α (+)	*P* value	p110β (+)	*P* value	*N*	p110α (+)	*P* value	p110β (+)	*P* value
Gender										
Male	35	25	0.450	30	0.101	36	28	0.580	27	0.332
Female	25	20		17		24	19		20	
Age (years)										
<60	30	22	0.766	22	0.347	28	20	0.184	21	0.392
≥60	30	23		25		32	27		26	
Tumor site										
Colon	47	37	0.205	39	0.097	40	33	0.164	32	0.448
Rectal	13	8		8		20	14		15	
Sizes (cm)										
<0.2	25	15	0.025※	12	0.000※	23	13	0.002※	14	0.012※
≥0.2	35	30		35		37	34		33	
Grade										
Low grade	29	21	0.440	22	0.445	30	23	0.754	23	0.754
High grade	31	24		25		30	24		14	
Number										
Single	40	28	0.206	35	0.015※	42	30	0.043※	30	0.043※
Multiple	20	17		12		18	17		17	

※Indicate statistical significant (*P*<0.05).

(P<0.05), and the number of both of adenomas was related to the expression of P110β (P<0.05) (**Table 3**). Nevertheless, no correlation was observed between p110α and p110β expression and clinicopathologic parameters in HP. In adenocarcinoma, p110α and p110β expression was correlated with the degree of tumor differentiation and lymph node metastasis (*P*<0.05) (**Table 4**).

Discussion

As premalignancy, colorectal adenomas often occur insidiously and are generally believed to have the potential of developing into adenocarcinomas [4, 5]. In recent years, endoscopes have facilitated the examination and diagnosis of colorectal adenomas, thus the detection rate of adenoma has risen. Nevertheless, early-stage precaution canceration of adenomas, therapy of adenomas and prognostic judgment are still primary in the prevention and treatment of neoplasms in digestive system. The conventional adenoma-carcinoma sequence is a gradually progressive transformation of normal mucosal epithelium tissue into adenoma and subsequent adenocarcinoma induced by a series of genetic changes [6]. While the "serrated neoplasia pathway", which has received more interests recently, also involves multiple genetic and epigenetic mutations, and abnormality in cellular signaling [7-11].

As discovered lately [12-14], unusual activation of PI3K/AKT signaling pathway can be detected during the occurrence and development of neoplasms. The pathway is critical in regulating growth, proliferation, survival and carcinogenesis of cells. Especially the correlation between the abnormal expression of p110α and p110β, which were encoded by PIK3CA and PIK3CB respectively, and the genesis and development of neoplasms have been extraordinarily concerned by researchers. Many papers on adenomas originated from different tissues have shown that occurrence of many carcinomas is related to the abnormal activation of PI3Ks. Viglietto et al. [15] found that 12% of thyroid neoplasm and 24% of thyroid carcinoma were accompanied by PIK3CA gene amplification. Lin et al. [16] have reported in a study that mutations on PIK3CA are involved in 9% of invasive pituitary adenoma, and reoccurrence of neoplasms is also closely related to PIK3CA abnormality. In the study previous to this paper [17], overexpressed p110α and p110β can be

Table 4. Correlation between p110α and p110β with different clinicopathologic parameters in colorectal adenocarcinoma

Parameters	*N*	p110α (+)	*P* value	p110β (+)	*P* value
Gender					
Male	56	42	0.293	42	0.293
Female	64	53		53	
Age (years)					
<60	51	40	0.865	41	0.776
≥60	69	55		54	
Tumor site					
Colon	68	54	0.940	53	0.705
Rectal	52	41		42	
Sizes (cm)					
<5	50	43	0.119	43	0.119
≥5	70	52		52	
Degree of differentiation					
High	58	40	0.008※	41	0.027※
Middle and low	62	55		54	
Depth of invasion					
No epicardial	31	24	0.781	23	0.429
Epicardial	89	71		72	
Lymph node metastasis					
No	32	20	0.007※	22	0.028※
Yes	88	75		73	
Type					
Tubular adenocarcinoma	40	32	0.785	30	0.592
Villous adenoma cancerations	40	31		32	
Serrated adenoma cancerations	40	32		33	

※Indicate statistical significant ($P<0.05$).

detected in colon carcinoma, and the genes are playing an important part in the development of the carcinoma. This study has shown that p110α and p110β are expressed differently in different stages of the canceration process. In conventional adenoma, p110α and p110β have increased as the normal mucosal tissues transform into low grade normal adenomas, then high grade normal adenomas and finally cancerated adenomas, but they showed no obvious change in high grade adenomas, villous adenomas of different grades and adenoma cancerations, all of which has revealed the close relativity between p110α and p110β genes and conventional adenoma-carcinoma sequence and the abnormal expression of p110α and p110β in early-stage VA. We believe that mutations of PIK3CA and PIK3CB genes are involved in the development of VA, and over expression of the genes is contributing to the evolution of high grade TA and TVA and different grade VA into adenocarcinoma as one possible initial factors of colorectal carcinomas.

Compared with normal adenomas, serrated adenomas bear the morphological characters of both adenomatous polyps and hyperplastic polyps and count for approximately 0.5% of all colorectal polyps. Despite their low morbidity rate, the canceration rate of serrated adenomas is higher than normal adenomas. Thus as a new type of premalignancy, the carcinogenesis mechanism is drawing more attentions of researchers. Researchers have shown that multiple mutations on Ras-MAPK signaling pathway have contributed to the occurrence and carcinogenesis of serrated lesions [18, 19], and activation of Wnt signaling pathway has been observed in the carcinogenesis of serrated lesions [20]. However, relativity of PI3K/AKT and "serrated neoplasia pathway" is seldom reported. The results of this study indicate that "serrated neoplasia pathway" is closely related to the over expression of p110α and p110β, the key molecules in PI3K/AKT signaling pathway. Expression levels of p110α and p110β are significantly higher in SSA, TSA and cancerated adenomas than in HP, and all groups are not significantly different, which indicates that PIK3CA and PIK3CB are among the mutated oncogenes in "serrated neoplasia pathway". We believe that mutations of PIK3CA and PIK3CB can possibly induce abnormal activation of PI3K/AKT signaling pathway, and promote the epithelial dysplasia of HP, and subsequently result in occurrence of SA. Since the abnormal activation starts at early-stage, it can prompt development and canceration since then. All these show the close relativity between PIK3CA and PIK3CB mutations and canceration of SA.

Canceration rates of different types of colorectal adenomas vary a lot. VA and SA are more

prone to canceration as shown in related research [21, 22]. Conventional adenomas and serrated lesions were comparatively investigated in this study, which revealed that over expressed p110α and p110β was discovered in VA and SA since the low grade stage, and the expression levels were not different in high grade adenomas and cancerated adenomas, indicating that p110α and p110β were overexpressed in VA and SA since the low grade stage whereas p110α and p110β levels were gradually increased in TA and TVA as the grading rose. Thus we believe that PIK3CA and PIK3CB mutations possibly contribute to not only the occurrence of VA and SA, but also the activation of the downstream signaling as the key molecules in PI3K/AKT pathway, resulting in abnormal proliferation of cells and suppressed apoptosis. Then p110α and p110β effectively promote the malignant transformation of VA and SA, leading to eventual canceration. Therefore, over expression of p110α and p110β is considered as one of critical factors in the increased malignant risk of VA and SA.

Expression of p110α and p110β is relevant to tumor size in all adenoma groups, and relevant to grade and amount of the adenomas in TVA. The expression levels of p110α and p110β are high in non-sessile VA. We have proposed a conjecture that mutations on PIK3CA and PIK3CB genes can promote the fast growth of adenomas, meanwhile these mutations in multiple sites and cells of mucous glands will lead to highly cancerated, large and multiple adenomas with flat sessile or no sessile. The expression of p110α and p110β is closely related to differentiation degree and lymph nodular metastasis in cancerated adenoma groups of both pathways, suggesting that p110α and p110β have critical influence on metastasis and prognosis of neoplasms, but the mechanism requires more investigation.

In summary, both “conventional adenoma-carcinoma” and “serrated neoplasia” pathways in colorectum have involved p110α and p110β overexpression which is possibly the risk factor of malignant transformation of adenomas. Especially in VA and SA, early-stage presence of over expressed p110α and p110β contributes to the significantly higher canceration rates of these adenomas comparing with other normal adenomas. Therefore, simultaneous detection of p110α and p110β is of great clinical importance in the early-stage prediction of canceration of adenomas and prognostic judgment of neoplasms.

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Disclosure of conflict of interest

None.

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