Over-Expression of the Overexpressed in Lung Cancer-1 Is Associated With Poor Prognosis in Colorectal Cancer

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Abstract. *Background/Aim: The overexpressed in lung cancer-1 (OLC1) protein is overexpressed in a variety of human tumors. The purpose of the present study was to determine whether increased expression of OLC1 is associated with colorectal cancer. Materials and Methods: OLC1 expression was assayed in 150 colorectal cancer tissues by immunohistochemical staining (IHC). Multivariate and univariate analyses were performed to determine the association between OLC1 expression and prognosis. Results: Immunohistochemical results revealed that 107 out of 150 colorectal cancer patients had increased levels of OLC1. OLC1 expression was significantly correlated with UICC stage (p<0.001) and histological differentiation (p<0.001) in colorectal cancer patients. The 5-year overall survival (OS) rates in patients with strong-positive and weak OLC1 staining were 16.6% and 95.3%, respectively (p<0.0001). Conclusion: OLC1 overexpression is an important factor in colorectal carcinoma prognosis and can be an interesting potential novel biomarker for colorectal cancer.*

Colorectal cancer (CRC) is one of the most common forms of human cancer worldwide, and it is the fifth most lethal malignancy in China, approximately one in ten malignancies are CRC (1). New research on CRC is determined by the constant increase in disease incidence both in developed and developing countries. Regrettably, due to the lack of effective biomarkers and early screening tools for CRC, when diagnosed, the tumor is most often at an advanced stage (2). Despite primary surgical or chemotherapy regimens, the 5-year survival rates for patients with advanced disease remain poor (3). There are numerous factors affecting CRC prognosis such as location, size, histological cancer type, degree of tumor invasion, loco-regional metastasis as well as metastasis in other organs. To improve CRC prognosis, basic research in genetics as well as in molecular biology are widely developed. Therefore, it is essential to discover effective biomarkers that could predict for prognosis and improve prognosis of patients with advanced CRC.

The overexpressed in lung cancer-1 (OLC1) protein is overexpressed in a variety of human tumors, and its highest expression is observed in lung cancer, immunohistochemical (IHC) staining has revealed that overexpression of OLC1 is detected in the most frequent types of lung cancers, as well as pulmonary pre-malignant lesions, it associated with cell apoptosis, cell-cycle progression (4). Furthermore, it has been demonstrated that the *OLC1* gene is associated with malignant transformation (5). The above publications immediately suggest that OLC1 may play important roles in the inception and progression of cancer. Therefore, OLC1 could be potentially used as a novel biomarker for CRC prognosis.

The present study intends to analyze the association between OLC1 and prognosis of CRC, we detected OLC1 expression in different phases of colorectal cancer by immunohistochemical analysis and explored the impact of OLC1 in colorectal cancer development.

Materials and Methods

Patients and tissue characteristics. Fresh colorectal carcinoma specimens, patient-matched adjacent colonic tissue and normal colonic tissue were collected from 150 colorectal cancer patients in the Third Affiliated Hospital of Harbin Medical University from January 2001 to December 2005. The distance between adjacent tissue and cancer tissue boundaries was approximately 1 cm, while that of distant normal tissue and cancer tissue was approximately 10 cm. All specimens were fixed in 10% formalin and embedded in paraffin.

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Key Words: Overexpressed in lung cancer 1 (OLC1), colorectal cancer, immunohistochemistry, prognosis.

Table I. *Expression of OLC1 in different colorectal tissues.*

	OLC1 Expression			*p*-Value
	OLC1(–/+) n(%)	OLC1(++) N(%)	OLC1(+++) N(%)	
Normal colonic tissue	135 (90.0%)	15 (10.0%)	0	<0.0001
Adjacent colonic tissue	88 (58.7%)	48 (32.0%)	14 (9.3%)	
Colorectal carcinoma tissue	43 (28.7%)	54 (36%)	53 (35.3%)	

Table II. *Relationship between OLC1 expression and clinicopathological factors in colorectal carcinoma.*

Characteristics	Sample size (n)	OLC1(-/+) n(%)	OLC1(++) n(%)	OLC1(+++) n(%)	*p*-Value
Age					
<55 year	53	17 (32.1%)	16 (30.2%)	20 (37.7%)	0.5393
≥55 year	97	26 (26.8%)	38 (39.2%)	33 (34.0%)	
Gender					
Male	85	23 (27.1%)	33 (38.8%)	29 (34.1%)	0.707
Female	65	20 (30.8%)	21 (32.3%)	24 (36.9%)	
Tumor location					
Colon	78	18 (23.1%)	27 (34.6%)	33 (42.3%)	0.1291
Rectum	72	25 (34.7%)	27 (37.5%)	20 (27.8%)	
Tumor size (cm)					
<5cm	95	29 (30.5%)	37 (39.0%)	29 (30.5%)	0.2682
≥5cm	55	14 (25.5%)	17 (30.9%)	24 (43.6%)	
Histological differentiation					
Well	19	15 (78.9%)	4 (21.1%)	0	<0.0001
Moderate	95	24 (25.3%)	42 (44.2%)	29 (30.5%)	
Poor/other	36	4 (11.1%)	8 (22.2%)	24 (66.7)	
UICC Stage					
Stage I	30	23 (76.7%)	7 (23.3%)	0	<0.0001
Stage II	44	16 (36.4%)	24 (54.6%)	4 (9.1%)	
Stage III	50	4 (8.0%)	16 (32.0%)	30 (60.0%)	
Stage IV	26	0	7 (26.9%)	19 (73.1%)	

All enrolled individuals gave their informed consent, and the Ethical and Scientific Committees of the hospital approved the study. None of the patients had undergone either chemotherapy or radiotherapy before surgery. Resected specimens were studied pathologically according to the criteria described in the International Union against Cancer (UICC) pTNM classification (2002). The patients details regarding age, sex, tumor location, tumor size, histological grade and stage are provided in Table I. The patients were followed-up for an interval of 12 to 72 months. All patients received chemotherapy with the majority receiving 5-fluorouracil and leucovorin. All deaths were attributable to colorectal cancer.

Immunohistochemistry. The folllowing polyclonal rabbit antibody was used for immunohistochemistry: Anti- OLC1 (Sanying Biotechnology Inc, Wuhan, China). Tissue sections were de-paraffinized with xylene, rehydrated through graded ethanol, and rinsed in phosphate-buffered saline (PBS). For antigen retrieval, the tissue sections were heated in a pressure cooker in citric acid monohydrate, pH 6.0, for 5 min. Slides were treated with 0.3% H_2O_2 for 30 min at room temperature to quench endogenous peroxidase activity, non-imunone serum albumin to block the non-specific binding, primary antibodies: anti-OLC1 (diluted 1:200) at 4˚C overnight. Then the tissue sections were treated with biotinylated secondary antibody, followed by further incubation with streptavidin–horseradish peroxidase complex. Immunoreaction was visualized with diluted DAB. Finally, the sections were counterstained with hematoxylin and examined microscopically. Negative control slides were stained with rabbit serum superseding primary antibodies. Positive controls were stained with prostate cancer whose OLC1 were strongly positive.

Evaluation of immunostaining. OLC1 was stained as buffy colored in the cytoplasm. The degree of immunostaining was reviewed and scored by two pathologists taking into account the percentage of positive cells and the staining intensity. The proportion of positively-stained tumor cells was scored as follows: 0 (no positive tumor cells), 1 (<10% positive tumor cells), 2 (10-50% positive tumor cells), and 3 (>50% positive tumor cells). Staining intensity was classified according to the following criteria: 0 (no staining), 1 (weak staining=light yellow), 2 (moderate staining=yellow brown), and 3 (strong staining=brown). Staining index was calculated as the staining intensity score × the proportion score. In randomly-chosen

Table III. *OLC1 is an independent prognostic factor by Cox regression analysis.*

Factor	Univariate analysis		Multivariate analysis	
	Hazard ratio (95%CI)	*p*1 value	Hazard ratio (95%CI)	*p*2 value
Age (years)				
<55 year	0.994 (0.606-1.631)	0.981	0.996 (0.580-1.713)	0.99
≥55 year				
Gender				
Male	1.166 (0.725-1.874)	0.529	1.133 (0.685-1.873)	0.629
Female				
Tumor location				
Colon	0.669 (0.414-1.081)	0.103	0.810 (0.485-1.353)	0.423
Rectum				
Tumor size (cm)				
<5 cm	1.385 (0.856-2.240)	0.187	1.175 (0.692-1.995)	0.553
≥5 cm				
Histological differentiation				
Well	2.685 (1.770-4.074)	<0.0001	0.850 (0.506-1.428)	0.5423
Moderate				
Poor/other				
Stage				
Stage I	3.118 (2.331-4.169)	<0.0001	2.428 (1.596-3.695)	<0.0001
Stage II				
Stage III				
Stage IV				
OLC1 expression				
OLC1(+–)	10.431 (6.266-17.365)	<0.0001	7.900 (4.466-13.973)	<0.0001
OLC1(++)				
OLC1(+++)				

cases, the immunostaining was performed in complete tissue sections to evaluate the consistency of the results.

Statistical analysis. Statistical studies were performed with Medcacl® version 10.4.3.0. The Chi-square test was used to evaluate the association between OLC1 expression and age, sex, tumor location, tumor size, histological differentiation, UICC stage. The Kaplan-Meier curves and log-rank test was used to determine the probability of survival. Correlations between OLC1 and clinical pathological factors were analyzed by calculating the Spearman's correlation coefficients. Univariate and multivariate statistical analysis were performed using the Cox regression analysis to study different variables and overall survival. $p<0.05$ was considered significant.

Results

OLC1 expression in colorectal cancer. A total of 150 cases were included in this study. Immunohistochemical staining determined whether OLC1 overexpression was associated with the clinical development from normal mucous to adjacent colonic tissue. Weak or rather absence of OLC1 expression was observed in all normal colorectal mucosa. OLC1 staining was observed to be gradually elevated in normal mucous compared to adjacent colonic tissue, and was highest in colorectal carcinoma (Table I).

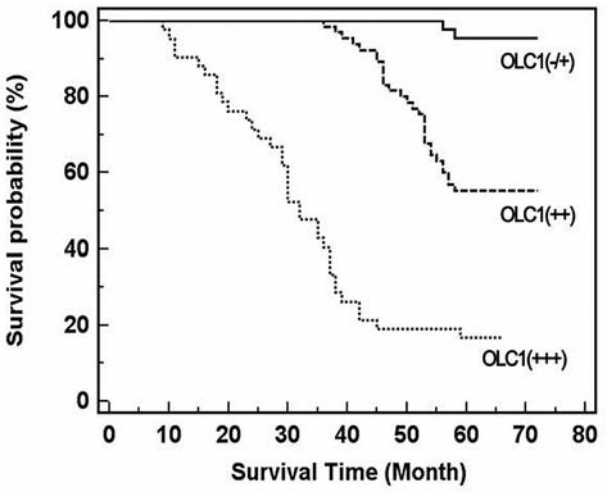

Figure 1. *Kaplan-Meier curves showing survival in 150 patients with colorectal cancer according to the categories of low-, moderate- and high-expression of OLC1 (analyzed with log-rank test).*

Relationship between OLC1 expression and the clinicopathological features of colorectal cancer. The correlation between the clinicopathological characteristics and OLC1 expression in colorectal carcinoma tissues are shown in Table

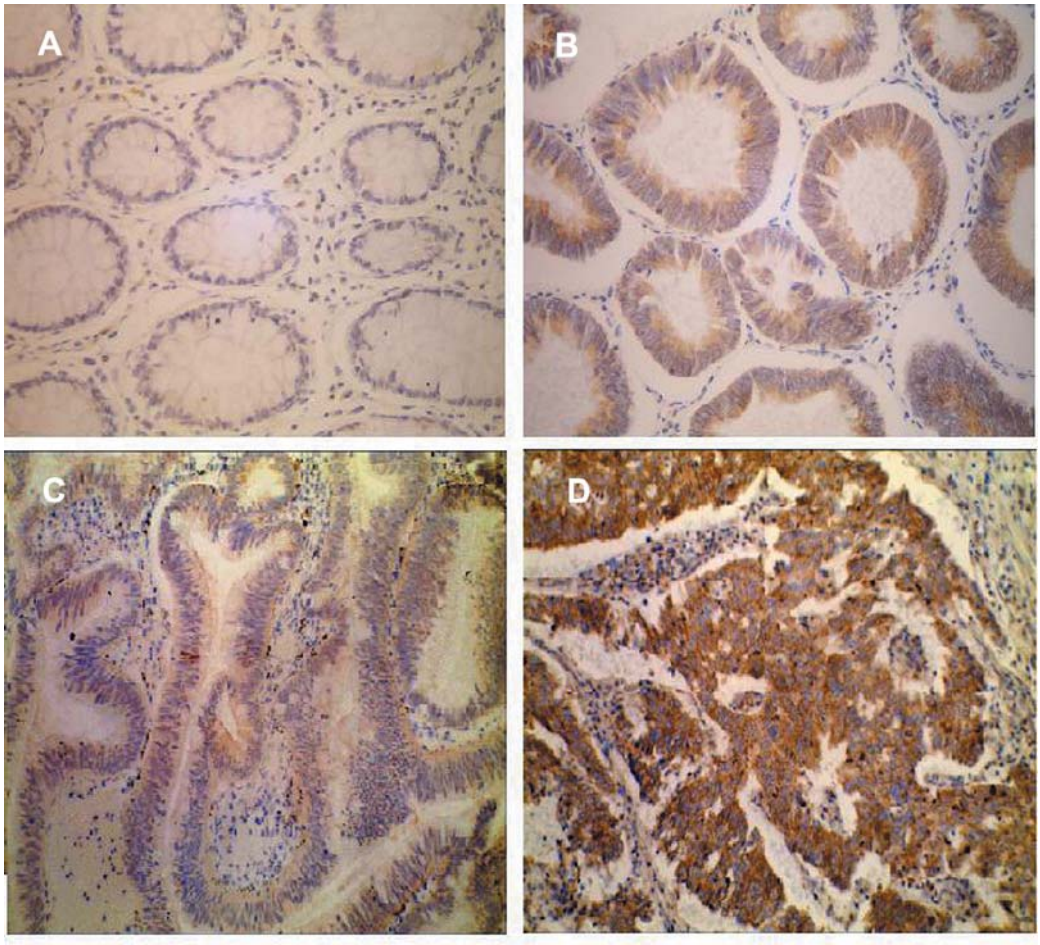

Figure 2. *Representative examples showing high, moderate, and low OLC1 expression. A: Normal ovaries, score: –; B: representative staining patterns of low OLC1 expression, score: +/–. C: Representative staining patterns of moderate OLC1 expression, score: ++; D: representative staining patterns of high OLC1 expression, score: +++. Original magnification, ×200.*

II. Out of 150 patients, 53 (35.3%) were younger than 50 years, 76 (50.67%) were stage III and IV. The tumor size after cytoreductive surgery was smaller than 5 cm is 95 (63.3%). The histological differentiation of the tumors included 36 cases (24%) of poor-, 95 cases (63.3%) of moderately-, 19 cases (12.7%) of well-differentiated colorectal carcinoma. According to immunohistochemical results, 43 (28.7%) out of the 150 colorectal cancer samples were categorized as exhibitting negative or weak staining (–/+). 54 (36.0%) and 53 (35.3%) were scored as exhibiting moderate-positive staining (++) and strong-positive staining (+++), respectively. OLC1 expression was correlated with UICC stage ($p<0.0001$) and histological differentiation ($p<0.0001$). However, OLC1 expression was not correlated with patient age, gender, tumor location and size.

Impact of OLC1 expression on overall patient survival. The mean patient follow-up time was 72 months. The cumulative 5-year overall survival rate of 150 patients was 54.7%. The cumulative 5-year survival rate was only 16.6% in the OLC1 (+++) group, whereas it reached 55.4% and 95.3% in the OLC1 (++) and OLC1 (–/+) protein expression group (Figure 1).

Both univariate and multivariate analyses showed that UICC stage and OLC1 protein overexpression were indepent poor prognostic factors of CRC. However, age, gender, tumor location, size and histological differentiation were not related to CRC prognosis (Table III).

Evaluation of immunohistochemical staining. The expression of OLC1 was assessed by immunohistochemical staining. Representative examples of OLC1 staining are shown in Figure 2. The characteristics of low-, moderate-, and high-expression categories are presented in Table II. One hundred fifty patients were studied. Fourty-three patients had low OLC1 expression, 54 had moderate expression, and 53 had

high expression. Seventeen patients in the low-expression group, 16 patients in the moderate group, and 20 patients in the high-expression group were younger than 55 years. In the low-expression group 23/30 (76.7%) patients were UICC stage I, 16/44 (36.4%) were stage II, 4/50 (8.0%) were stage III and there was no UICC stage IV patient. In the moderate-expression group, 7/30 (23.3%) patients were UICC stage I, 24/44 (54.6%) were stage II, 16/50 (32%) were stage III and 7/26 (26.9%) were UICC stage IV. In the high-expression group, there was no patient of UICC stage I, 4/44 (9.1%) patients were UICC stage II, 30/50(60%) were stage III and 19/26(73.1%) were stage IV. There were no statistically significant differences in patients' characteristics such as age, gender, tumor size and tumor location ($p>0.1$) between the low-, moderate-, high-expression group. However, there were significant differences in histological differentiation and UICC stage ($p<0.0001$ and $p<0.0001$, respectively).

Discussion

The results from this large, prospective cohort study of colorectal cancer demonstrate that OLC1 is overexpressed in colorectal carcinomas, and correlates well with patients' poor prognosis. OLC1 could serve as a clinically-relevant indicator of early diagnosis as well as a prognostic biomarker for colorectal carcinoma.

Over the last years colorectal cancer has become the third most common cancer in the world. Due to changing of lifestyle, increase in obesity and physical inactivity, colorectal cancer is more and more popular in China (6). Colorectal cancer recognizes a natural evolution compatible to a long asymptomatic period, corresponds to early stage of cancer transformation of adenomatous polyps to cancer and invasion beyond the basement membrane (7). The diagnosis of CRC is most often performed when the tumor is in advanced stages, when the extent of lesions and their location are determinants of clinical expression and invasion beyond the basal lamina (8). To improve CRC prognosis, research in genetics and molecular biology is immensely implemented. Unfortunately, there is no effective biomarkers for the prognosis of colorectal cancer.

OLC1 was first discovered in lung cancer, and can be detected in almost all lung cancer types, it can also be detected in different stages of the disease, including typical hyperplasia and carcinoma stage (9). Furtherore research has shown that OLC1 overexpression plays an important role in human lung tumorigenesis and is suggested to be a potential early diagnostic and therapeutic target for the specific disease type (10). In our study, we examined the expression of OLC1 in colorectal cancer tissues, adjacent colonic tissues and normal colonic tissues. We showed that OLC1 is overexpressed in cancer tissues and it is absent from almost all normal tissues. Furthermore, we found that the expression of OLC1 is increasing as the aggravating of cancer. Statistical analyses showed that OLC1 expression significantly correlates with histological differentiation and UICC stage, but has no correlation with patients' age, sex, tumor location or tumor size. It is interesting that our study showed that the stronger OLC1 presence, the poorer was the patients' prognosis. The 5-year survival rate was only 16.6% in the high OLC1 (+++) group and 55.4% in the moderate group (++), whereas it reached 95.3% in the low group (–/+). These results demonstrated that OLC1 might not only be used as a predictor of early diagnosis but also as a prognostic biomarker for colorectal cancer.

To date, the association between OLC1 and prognosis in CRC have not been reported. This is the first study using a large number of clinical samples to analyze the effect OLC1 as a prognostic biomarker for CRC. Our preceding results have shown that overexpression is an important factor in epithelial ovarian carcinoma and can be a potential biomarker for ovarian cancer. It is demonstrated that OLC1 might be used as an early warning sign of cancer. As known OLC1 is an activater of NF-kB reportor, and NF-kB is a tumor promoter in inflammation-associated cancer (11, 12). It is interesting that activation of NF-kappaB is an early event during carcinogenesis and NF-kB signal pathway can regulate the expression of many genes involved in tumorigenesis, cancer progression and invasion (13, 14). Human bronchial epithelial cells (MBE) with OLC1 overexpression showed different colony formation activity, increased growth rate, and cell-cycle progression (15, 16). It is generally known that the NF-kB pathway plays an important role in cancer invasion and metastasis (17, 18). Thus, we supposed that OLC1 is associated with the development of CRC. In the present study, we analyzed OLC1 expression in 150 patients with CRC and found that OLC1 expression was an important factor for prognosis.

In summary, through the present study it is shown that OLC1 may serve as a clinically-relevant indicator of early diagnosis and prognostic biomarker for colorectal carcinoma. Moreover, further *in vitro* and *in vivo* experiments should be conducted to elucidate the underlying mechanism of OLC1 and the viability of OLC1 as a therapeutic target in human tumors.

Conflicts of Interest

The Authors declare that they have no conflicts of interest.

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