Int J Clin Exp Pathol 2014;7(11):8077-8081
www.ijcep.com /ISSN:1936-2625/IJCEP0001826

Original Article

Decreased expression of interleukin-36α predicts poor prognosis in colorectal cancer patients

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Received August 11, 2014; Accepted September 13, 2014; Epub October 15, 2014; Published November 1, 2014

Abstract: Interleukin-36α (IL-36α), previously designated as IL-1F6, has been found to have a pathogenic role in psoriasis. However, possible functions of IL-36α in cancer remain unclear. In present study, we investigate the possible role of interleukin-36α involved in the pathogenesis of colorectal cancer. IL-36α expression was detected in 345 colorectal cancer tissue samples by immunohistochemical staining, and its relation with clinicopathologic parameters and prognosis of colorectal cancer patients were analyzed. IL-36α was highly expressed in nearly half of all tested colorectal cancer patients. However, low expression level of IL-36α significantly correlated with larger tumor size and advanced TNM stage. Kaplan-Meier survival analysis showed that low expression level of IL-36α resulted in a remarkably poor prognosis of colorectal cancer patients. Multivariate Cox's analysis revealed that the IL-36α expression level was a significant and independent prognostic factor for overall survival rate of colorectal cancer patients. Thus, our study may provide insight into the application of IL-36α as a novel predictor of prognosis and a potential therapeutic drug for colorectal cancer.

Keywords: Interleukin-36α, colorectal cancer, prognosis, therapy

Introduction

Colorectal cancer is one of the most common malignancies with millions of new cases worldwide each year [1]. Despite many improvements have made in diagnostic procedures and therapeutic strategies, the clinical outcome and prognosis for colorectal cancer patients are still unsatisfactory [2]. Thus, similar to other cancers, it's urgent to understand the underlying mechanisms involved in the progression of colorectal cancer for development of more effective therapies to clinically manage this disease.

IL-36 is a member of newly identified IL-1 family that included IL-36α, IL-36β and IL-36γ [3]. Similar to the functions of IL-1 cytokines, IL-36 cytokines are also involved in immune responses [4]. And different from the situation of IL-1 expression pattern, IL-36 cytokines are predominantly expressed in the skin and epithelial tissues [5-7]. Increased IL-36α expression has been detected in psoriatic skin, which indicates that IL-36α cytokines may exhibit a pro-inflammatory role in this disorder [8-10]. Despite the majority of studies focus on inflammatory disease, the possible role of IL-36α in the pathogenesis of cancer remains unknown.

Therefore, the current study were done to characterize the role of IL-36α in colorectal cancer by investigating the IL-36α protein expression profile in 345 primary tumor surgical specimens from colorectal cancer patients. The results indicate that IL-36α expression was decreased during malignant transformation in colorectal cancer and suggest that the low expression level of IL-36α is a reliable indicator for the poor prognosis of colorectal cancer patients.

Materials and methods

Clinical tissue samples

Consecutive patients with colorectal cancer were recruited from January 2003 to November 2010 at Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University. The cases of colorectal cancer were selected in this study

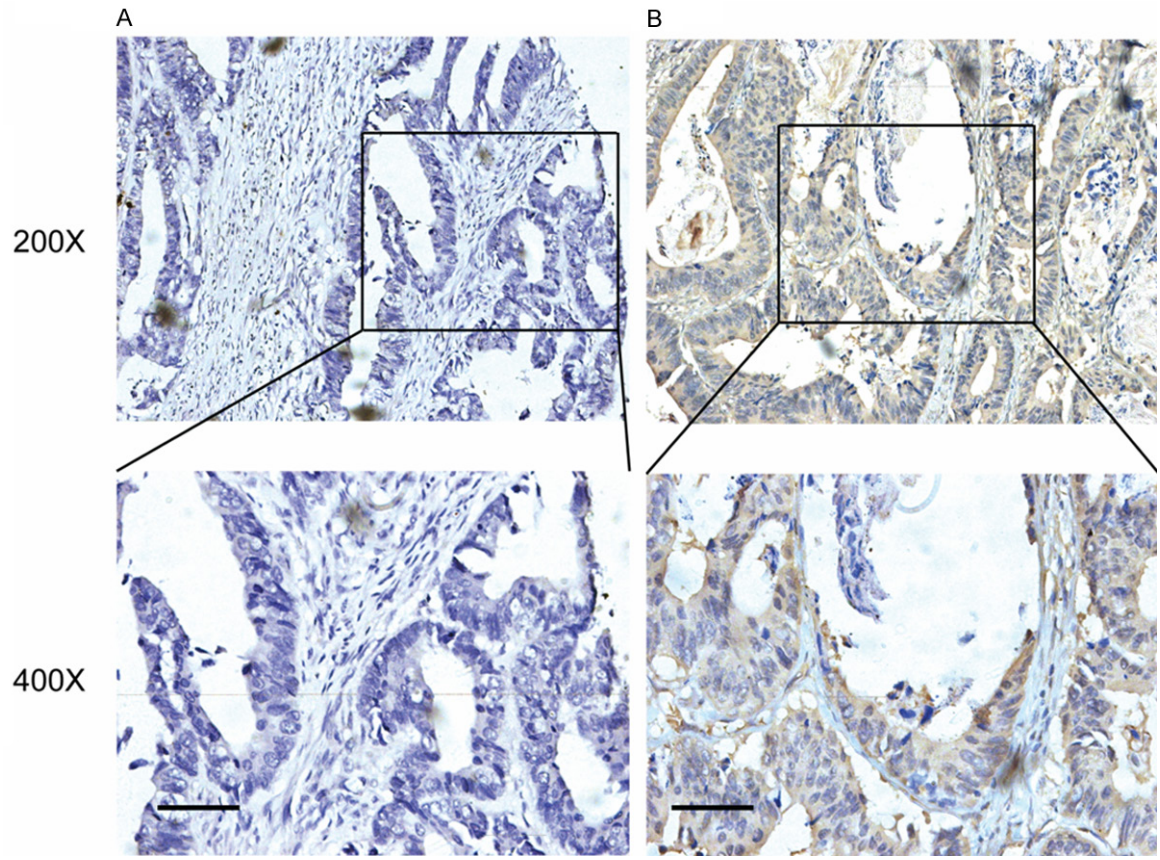

Figure 1. IL-36α expression in colorectal cancer tissues. A: Negative expression of IL-36α; B: Positive expression level of IL-36α. Representative images are shown at × 200 and × 400 magnifications, respectively. Scale bar: 50 μm.

Table 1. Relationship between IL-36α expression and clinicopathological features in colorectal cancer patients

Variable		IL-36α (n)		
		Low	High	*P*
		n = 184	n = 161	
Age	≤ 65 years	100 (53.48)	87 (46.52)	0.954
	> 65 years	84 (53.16)	74 (46.84)	
Gender	Male	105 (52.76)	94 (47.24)	0.804
	Female	79 (54.11)	67 (45.89)	
Tumor size	≤ 5 cm	78 (44.83)	96 (55.17)	**0.001**
	> 5 cm	106 (61.99)	65 (38.01)	
Tumor location	Rectum	106 (52.48)	96 (47.52)	0.704
	Colon	78 (54.55)	65 (45.45)	
TNM stage	0-I	22 (25.88)	63 (74.12)	**0.000**
	II	71 (59.66)	48 (40.34)	
	III	73 (62.39)	44 (37.61)	
	IV	18 (75.00)	6 (25.00)	
Histology	Mucinous	23 (42.59)	31 (57.41)	0.085
	Non-mucinous	161 (55.33)	130 (44.67)	

Values in parentheses indicate percentage values. The bold number represents the *P*-values with significant differences.

only if clinical data were available. The follow-up time was calculated from the date of surgery to the date of death, or the last known follow-up. None of them had received radiotherapy, chemotherapy, hormone therapy or other related anti-tumor therapies before surgery. Five tissue microarrays containing 345 human colorectal cancer tissue samples were enrolled in this study.

Immunohistochemical staining

Tissue sections were deparaffinized in xylene and rehydrated with graded ethanol. The sections were incubated with 0.3% hydrogen peroxide

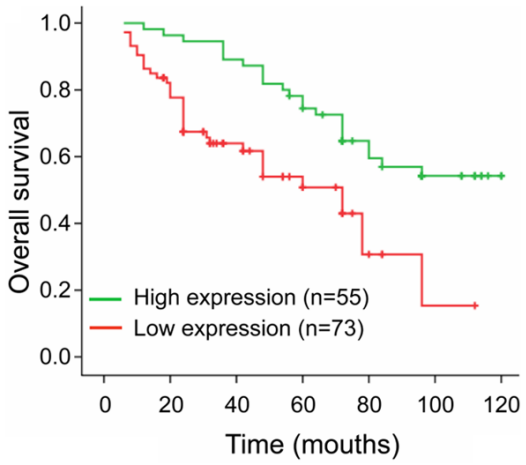

Figure 2. Kaplan-Meier curves for patients grouped based on IL-36α expression. The overall survival rate of colorectal cancer patients was significantly decreased in low IL-36α expression group compared with high IL-36α expression group (P = 0.001). Time is shown in mouths.

and boiled in a microwave for 15 min to unmask antigen epitopes and then blocked with 10% BSA. After rinsing three times with phosphate-buffered saline (PBS), slides were first incubated using the antibody for IL-36α (Abcam, US) at 4°C overnight with optimal dilution. After washing three times with PBS, slides were incubated with second antibody labeled by HRP (rabbit) (Proteintech, US) at room temperature for 1 hour. Finally, the bound antibodies were visualized with 3,3'-diaminobenzidine tetrahydrochloride and counterstained by hematoxylin. Scoring was conducted according to the percent of positive cells: < 5% scored 0; 6%-25% scored 1; 25%-50% scored 2; more than 50% scored 3 and staining intensity: no staining scored 0, weakly staining scored 1, moderately staining scored 2 and strongly staining scored 3, respectively. The final score was designated as low or high expression group using the percent of positive cell score × staining intensity score as follows: low expression was defined as a total score < 4 and high expression with a total score ≥ 4. These scores were determined independently by two senior pathologists.

Statistical analysis

All statistical analyses were performed using the SPSS 16.0 software. Correlation of IL-36α expression with clinicopathologic parameters was analyzed by Pearson chi-square test. Overall survival rate was calculated according to the Kaplan-Meier method and the difference in survival curves was evaluated by the log-rank test. Independent prognostic factors were analyzed by the Cox proportional hazards regression model. $P < 0.05$ was considered statistically significant.

Results

Expression of IL-36α in human colorectal cancer

To observe the expression change happened in colorectal cancer, we first tested the expression level of IL-36α protein in 345 colorectal cancer tissue samples as demonstrated by immunohistochemical staining. The immunoreactivity of IL-36α was mainly distributed in the cytoplasm of colorectal cancer cells (**Figure 1**). Overall, IL-36α was low (**Figure 1A**) and high (**Figure 1B**) expressed in 184 (53.33%) and 161 (46.67%) of the 345 colorectal cancer samples, respectively (**Table 1**).

Correlation between IL-36α expression and clinicopathological parameters

The relationship between the IL-36α expression level and corresponding clinicopathological parameters of the colorectal cancer patients was calculated by Pearson chi-square test. As shown in **Table 1**, low expression of IL-36α was significantly associated with adverse clinicopathological features of colorectal cancer, including tumor size and TNM stage. No significant difference was found in age, gender, tumor location and histology.

Correlation between IL-36α expression and patient survival

To investigate the prognostic effect of IL-36α, the overall survival rate of colorectal cancer patients was analyzed using Kaplan-Meier survival curves and the log-rank test. The result revealed that low expression level of IL-36α protein was a significant prognostic factor for poor overall survival (OS) of colorectal cancer patients. As shown in **Figure 2**, a significant difference was observed on the Kaplan-Meier survival curves for colorectal cancer patients with a high or a low expression level of IL-36α. Univariate Cox regression analysis showed that IL-36α expression level and TNM stage are the

Table 2. Univariate and multivariate analysis showing the overall survival in colorectal cancer

Variable	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P* value	HR	95% CI	*P* value
IL-36α	0.395	0.227-0.686	**0.001**	0.519	0.290-0.928	**0.027**
Age	0.617	0.367-1.038	0.069			
Gender	0.895	0.532-1.506	0.676			
Tumor size	1.209	0.720-2.029	0.472			
Tumor location	0.804	0.473-1.367	0.420			
TNM stage	1.804	1.368-2.379	**0.000**	1.647	1.230-2.206	**0.001**
Histology	0.888	0.461-1.714	0.724			

HR: Hazard ratio; CI: Confidence interval. The bold number represents the *P*-values with significant differences.

significant risk factors for OS (**Table 2**). The relative risk was 0.395 for patients with a low IL-36α expression level compared with those with a high IL-36α expression level. Multivariate Cox regression analysis revealed that IL-36α expression was an independent factor in prediction of the overall survival rate of colorectal cancer patients (**Table 2**).

Discussion

In several types of human cancers, IL-1 has been reported that associate with virulent tumor phenotype [11-13]. Although belong to the IL-1 family as a result of the sequence homology, IL-36α has been demonstrated to play a crucial in skin inflammatory diseases, especially psoriasis [14]. Therefore, it is reasonable to suppose that IL-36α may play a role in the pathogenesis of cancer.

In the current study, low IL-36α expression accounts for 53.33% of the 345 colorectal cancer samples, which is consistent with the reported data in human hepatocellular carcinoma, indicating that dysregulated IL-36α expression may be involved in the progression of colorectal cancer [15]. By analyzing the relationship between IL-36α expression and corresponding clinicopathological parameters, we found low IL-36α expression was significantly correlated with larger tumor size and advanced TNM stage. Kaplan-Meier analysis revealed that the prognosis of colorectal cancer patients with a low expression level of IL-36α was poor and Cox regression analysis indicated that low expression level of IL-36α was an independent prognostic factor for a poor OS in colorectal cancer patients. These results above suggest that IL-36α may exhibit an anti-cancer role in the progression of colorectal cancer. It has been reported that tumor tissues with high IL-36α expression exhibited a significantly higher proportion of intratumoral $CD3^+$ and $CD8^+$ tumor-infiltrating lymphocytes (TILs), but not $CD4^+$ TILs [15]. This indicates that IL-36α could recruit $CD3^+$ and $CD8^+$ TILs and activate the adaptive T cell immune response, which ultimately determines the prognosis of patients of colorectal cancer.

In conclusion, the prognosis of patients with a low expression level of the IL-36α protein is poor, which may be attributed to the relation between IL-36α and colorectal cancer immune response. Current evidence identifies IL-36α as a potential anti-cancer therapy for colorectal cancer.

Acknowledgements

This work was supported by the grant from Science and Technology Commission of Shanghai, China (no.13DZ1942806).

Disclosure of conflict of interest

None.

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