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Clinical and prognostic significance of Krüppel-like transcription factor 6 expression in 67 patients with cutaneous malignant melanoma in China

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Krüppel-like transcription factor 6 (KLF6) is a ubiquitous tumor suppressor gene involved in regulating cell growth, proliferation, differentiation and angiogenesis. The objective of the study was to investigate the clinical and prognostic significance of KLF6 expression in cutaneous malignant melanoma (CMM) patients. A total of CMM 67 patients were enrolled in this study. The specimens were evaluated by immunohistochemistry to detect KLF6. The positive KLF6 expression in CMM tissues was significantly lower than in normal skin tissues ($p < 0.01$). The presence of KLF6 in CMM was correlated with ulceration ($p < 0.01$), lymph node metastasis ($p < 0.01$) and clinical stage ($p < 0.01$). The overall 5-year survival rate of the 67 patients was 13.4%. The 5-year survival rate of patients with negative KLF6 expression was correlated with KLF6 expression ($p < 0.01$), ulceration ($p < 0.01$), lymph node metastasis ($p < 0.01$), clinical stage ($p < 0.01$) and operation type ($p < 0.01$). Ulceration and clinical staging were independent relevant factors. In conclusion, the presence of KLF6 in CMM was correlated with ulceration, lymph node metastasis, clinical stage and poor prognosis.

Key words: KLF6, cutaneous malignant melanoma, prognosis, immunohistochemistry.

Introduction

Cutaneous malignant melanoma (CMM) is a common malignant tumor in western countries and is known for its rapid progression, metastasis, and poor prognosis [1]. It is relatively rare in Asians. In recent years, CMM has been one of the most rapidly increasing cancers in China [2]. The TNM staging system is widely used to guide therapeutic decisions and prognosis in patients with CMM in the current practice. However, it often could not supply the precise outcome to the individual patient. Recently some molecular biomarkers combined with the TNM staging system might be valuable to accurately distinguish CMM patients' prognosis.

The Krüppel-like transcription factor 6 (KLF6) is a member of the Krüppel-like factor family. It belong to a group of zinc finger transcription factors [3, 4]. Recent studies show that KLF6 is a ubiquitous tumor suppressor gene involved in regulating cell growth,
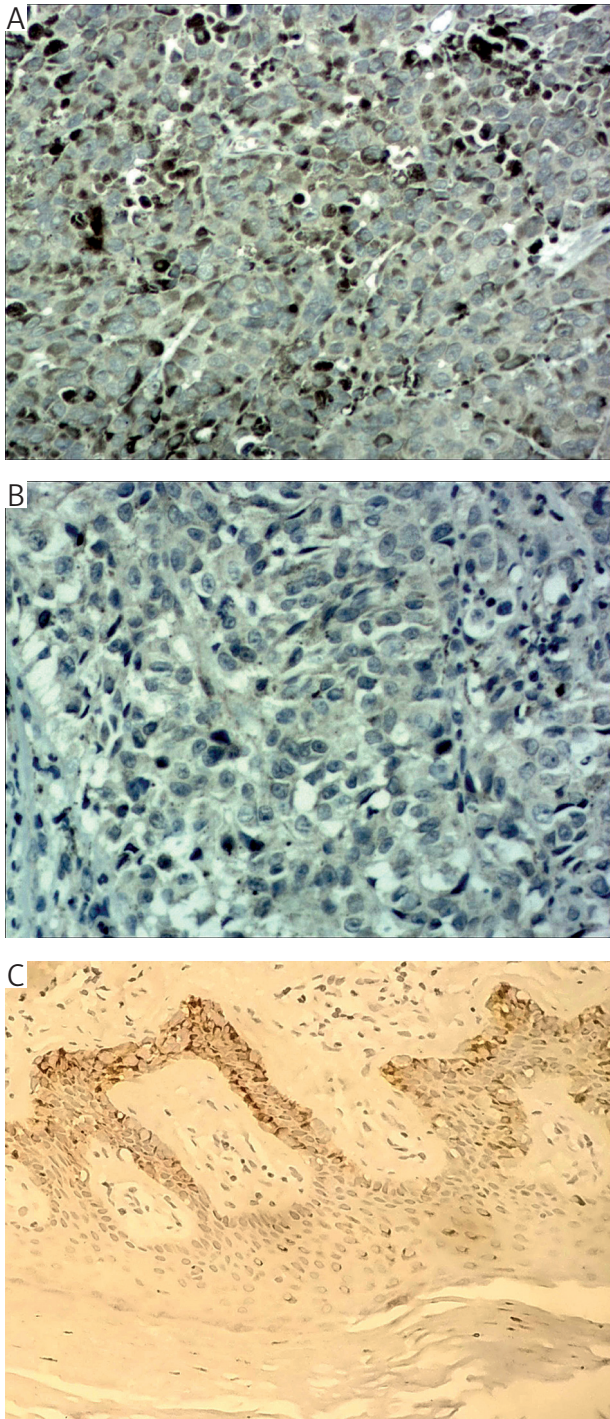
Fig. 1. A) Immunohistochemical staining of human CMM tissue sections demonstrating KLF6 protein. The KLF6 staining was mainly located in the cytoplasm, and photomicrographs showed human CMM specimen (foot) with high KLF6 expression tumor cells (≥ 7). Original magnification ×200; B) Photomicrographs showing CMM specimen (foot) with low KLF6 expression tumor cells (≤ 6). Original magnification ×200; C) Photomicrographs showing the corresponding normal tissue specimen (neck) with high KLF6 expression normal cells (≥ 7). Original magnification ×200

proliferation, differentiation and angiogenesis. It is closely associated with the occurrence of prostate cancer, liver cancer, lung cancer, etc. [5, 6, 7]. It is also reported that KLF6 is an important prognostic factor in some cancers [8]. There has been limited research confirming the correlation between KLF6 expression and clinic pathological value in CMM. Thus, we designed the present study to investigate the clinical and prognostic significance of KLF6 expression in CMM patients by both univariate and multivariate analysis. Also we detected KLF6 expression by immunohistochemistry.

Material and methods

Patients

A total of 67 patients were enrolled in this study at the Department of Dermatology, Department of Oncology, Jinan Central Hospital Affiliated to Shandong University and the Department of Dermatology, Qilu Hospital between January 2001 and December 2011. The inclusion criteria were as follows: (1) the pathology confirmed CMM by surgery or biopsy; (2) TNM staging according to the American Joint Committee on Cancer (AJCC) staging system (6th edition); (3) patients accepted no preoperative radiotherapy or chemotherapy; (4) the cases have been well preserved. The clinicopathological characteristics of the 67 patients are listed in Table I. This study was approved by Shandong University Ethics Committee.

Immunohistochemistry

All the CMM specimens were obtained from the 67 patients. The 10 control specimens were obtained from areas of normal skin tissue, taken from the patients who underwent surgery (healthy tissue sites were 3 cm away from the CMM tissue).The tissue specimens were fixed in 10% neutral buffered formalin and processed routinely. Hematoxylin and eosin (HE)-stained slides as well as immunohistochemical reactions were performed on paraffin-embedded and formalin-fixed tissue using rabbit antihuman KLF6 monoclonal antibodies (Abcam, Cambridge, UK; 1 : 100 dilution) and visualized by the Envision System (Dako). The presence of KLF6 was determined by light microscopy (BX51, Olympus Optical, Tokyo, Japan). KLF6 was mainly located in the tumor cell cytoplasm. KLF6 protein expression is indicated by yellow-to-brown color and was scored by the semiquantitative immunoreactivity scoring system (IRS), as follows: staining intensity (0 = no staining, 1 = weak staining, 2 = moderate staining, 3 = strong staining) and the percentage of cells stained (0 = no cells, 1 = 1-10% of cells, 2 = 11-50%

Table I. Correlation between KLF6 expression and clinical features of the CMM patients

CLINICAL CHARACTERISTICS	PATIENT	KLF6 EXPRESSION		P VALUE[A]
		LOW	HIGH	
	67	50	17	
Gender				0.159*
Male	36	24	12	
Female	31	26	5	
Age, years				1.000*
< 50	33	25	8	
≥ 50	34	25	9	
Locality				> 0.05
Head and neck	6	4	2	
Trunk	10	8	2	
Extremities	51	38	13	
Ulceration				0.001*
Yes	26	13	13	
No	41	37	4	
Histological type				> 0.05
NM	12	11	1	
SSM	49	36	13	
ALM	6	3	3	
pN				0.004*
−		26	13	13
+		41	37	4
Clinical stage				< 0.01
I		4	0	
II		16	9	
III		33	28	
IV		14	13	

*P Value[a]: χ^2 test, *Fisher's exact probability test*

of cells, 3 = 51-80% of cells, 4 = 81-100% of cells) were evaluated and the respective scores were multiplied, resulting in an IRS range from 0 to 12. For statistical analysis, cases were grouped as either low expression (IRS 0-6) or high expression (IRS 7-12), according to previous reports [9].

Statistical methods

Fisher's exact probability test or the χ^2 test was used to calculate the correlation between KLF6 expression and clinicopathological factors. Univariate analysis was carried out by the use of the Kaplan-Meier survival curves. Multivariate analysis was analyzed using the Cox proportional hazard model. When the p value was less than 0.05, we considered
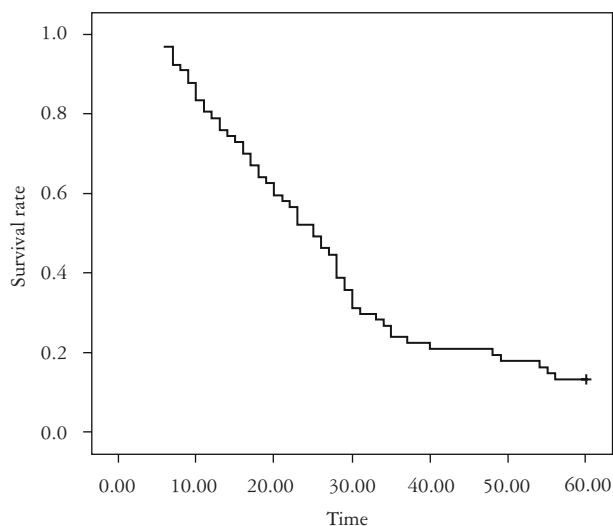
Fig. 2. Kaplan-Meier analysis of overall survival
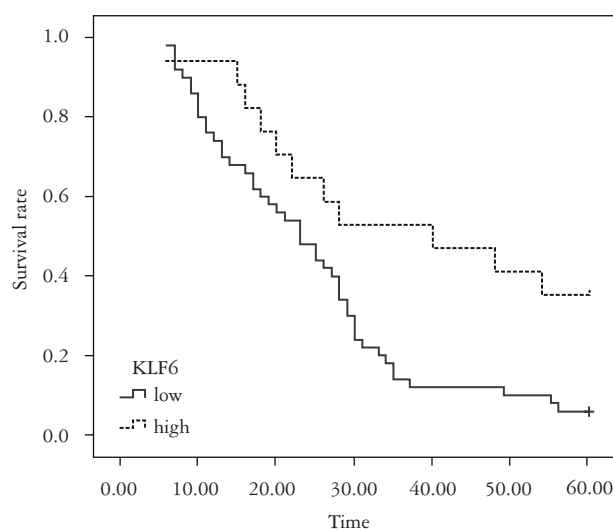
Fig. 3. Kaplan-Meier analysis of overall survival rate in patients with KLF6 expression

the differences significant. The obtained data were analyzed using the statistical software package SPSS (SPSS 13.0, SPSS Inc., Chicago, IL, USA).

Results

The protein KLF6 was found in normal tissues and malignant melanoma lesions: positive staining of a yellow-to-brown color was mainly observed in the epithelial prickle and basal layers in normal tissues; in malignant melanoma lesions, yellow staining was observed in the cuticular layer. CMM tissue from 17 patients was positive for KLF6, giving a positive rate of 25.4%, which was significantly lower compared with control tissues (p < 0.01) (Fig. 1). The presence of KLF6 in CMM was correlated with ulceration (p < 0.01), lymph node metastasis (p < 0.01) and clinical stage (p < 0.01). There was

Table II. Results of univariate survival analysis of the CMM patients

Clinical characteristics	Patients	5-year survival (%)		P value
	67	Patients	Rate(%)	
		9	13.4	
Gender				0.080
Male	36	8	22.2	
Female	30	1	3.3	
Age (years)				0.316
< 50	33	4	12.1	
≥ 50	34	5	14.7	
Locality				0.361
head and neck	6	1	16.7	
trunk	10	1	10.0	
extremities	51	7	13.7	
Ulceration				0.000
yes	41	0	0	
no	26	9	34.6	
Histological type				0.171
NM	12	1	8.3	
SSM	6	2	33.3	
ALM	49	6	12.2	
pN				0.000
−	26	9	34.6	
+	41	0	0	
Clinical stage				< 0.01
I	4	3	75.0	
II	16	6	37.5	
III	33	0	0.0	
IV	14	0	0.0	
Operative type				0.000
Surgery	46	9	19.6	
Biopsy	21	0	0.0	
Chemotherapy				0.637
Yes	23	5	21.7	
No	44	4	9.1	
Radiotherapy				0.707
Yes	8	2	25.0	
No	59	7	11.9	
Immunotherapy				0.809
Yes	53	7	13.2	
No	14	2	14.3	
KLF6 expression				0.008
Low	50	3	6.0	
High	17	6	35.3	

P values were obtained with the log-rank test

Table III. Results of multivariate Cox regression analysis of survival

	B	SE	Wald	P	HR	95% CI for HR
Gender	−0.003	0.317	0.000	0.992	0.997	0.535-1.856
Age	−0.179	0.151	1.408	0.235	0.836	0.623-1.124
Locality	0.523	0.275	3.612	0.057	1.687	0.984-2.894
Ulceration	0.983	0.464	4.495	0.034	2.672	1.077-6.630
Histological type	0.136	0.195	0.487	0.485	1.146	0.781-1.681
pN	0.036	0.486	0.005	0.941	1.037	0.400-2.689
Clinical stage	1.411	0.375	14.173	0.000	4.102	1.967-8.553
Operative type	−0.202	0.346	0.339	0.561	0.817	0.415-1.612
Chemotherapy	−0.506	0.388	1.695	0.193	0.603	0.282-1.291
Radiotherapy	−0.676	0.481	1.972	0.160	0.509	0.198-1.306
Immunotherapy	0.095	0.383	0.061	0.805	1.099	0.518-2.330
KLF6 expression	0.036	0.486	0.005	0.941	1.037	0.400-2.689

B – regression coefficient; SE – standard error; Wald – Wald value; HR – hazard ratio; CI – confidence interval

no significant correlation between KLF6 and other clinicopathological features (Table I).

Among the CMM patients, 46 patients had undergone surgery, 23 patients had adjuvant chemotherapy, 8 patients had adjuvant radiotherapy and 53 patients had immunotherapy. The overall 5-year survival rate of the 67 patients was 13.4% (Fig. 2). In univariate analysis by the log-rank test, the 5-year survival rate of patients with negative KLF6 expression was correlated with KLF6 expression (p < 0.01), ulceration (p < 0.01), lymph node metastasis (p < 0.01), clinical stage (p < 0.01) and operation type (p < 0.01; Fig. 3). There was no significant difference among patients according to gender, age, tumor locality, histological type, chemotherapy, radiotherapy or immunotherapy (Table II). The results of Cox regression multivariate analysis showed that ulceration and clinical staging were independent relevant factors (Table III).

Discussion

The *KLF6* gene is regarded as a tumor suppressor on the basis of its inactivation by somatic mutations and loss of heterozygosity in prostate malignant tumor [10, 11]. Recently, some reports showed that the *KLF6* gene could also be inactivated in many cancers [12, 13]. The studies reported that the growth suppressive properties of KLF6 were associated with some cancer pathways, including up-regulating p21 and E-cadherin, down-regulating cyclin D1and CDK4, etc. [14, 15]. However, there are few reports in the literature investigating *KLF6* mutations in CMM. Cai reported that only 27.5% of patients had positive expression of KLF6 in CMM tissues [16]. To further clarify the role of KLF6 in CMM, we examined expression of KLF6 in 67 CMM samples by immunohistochemistry. In the study, pos-

itive expression of KLF6 in CMM was only found in 17 cases (25.4%) and was remarkably lower than expression in normal skin tissue. Statistically, decreased expression of KLF6 was significantly associated with ulceration, lymph node metastasis and clinical stage in CMM. Our results suggested that KLF6 might be involved in tumor development and progression in CMM. However, few studies have reported the clinical and prognostic significance of KLF6 in cancer patients. In Cai's study, the univariate survival analysis showed that the 3-year survival rate in CMM patients with low levels of KLF6 protein was significantly lower than that in patients with high levels of KLF6 (p < 0.05) [16]. In our study the overall 5-year survival rate of the 67 CMM patients was 13.4%, and the 5-year survival rate of CMM patients with reduced KLF6 expression was significantly lower than that of patients with positive KLF6 expression, although it was not an independent relevant factor for prognosis.

It has been reported that some clinical characteristics of CMM patients were significantly different among different ethnic groups [17]. Our study showed that 61.2% of the patients had ulcers at the time of the initial diagnosis. Compared with other races, the primary tumor site is more likely to be an ulcer in the initial diagnosis of Asians [18]. Ulceration has previously been shown in a multivariate analysis to be an independent prognostic indicator of disease-free survival in melanoma patients [19]; but in a study by van Akkoi *et al*., it did not remain an independent prognostic factor for overall survival after the multivariate analysis [20]. In our study, the 5-year survival rate of patients with ulceration was significantly lower than that of patients without ulceration. Cox regression multivariate analysis

showed that ulceration was also one of the independent relevant factors for prognosis.

Stage has been repeatedly confirmed to be one of the most important prognostic indicators for melanoma [21, 22]. However, most evidence presented in the literature originated in endemic regions, particularly western countries, and the most commonly diagnosed subtype of malignant melanoma is superficial spreading melanoma (SSM). Due to the high prevalence of the disease, knowledge on diagnosis and screening is readily available and diagnoses are usually made in relatively earlier stages. Cascinelli reported that the 5-year survival rate of the patients was 80.8% in the white race in western countries [23]. Our study showed only 29.8% patients with stage I-II, and the 5-year survival rate in patients was significantly associated with lymph node metastasis and clinical staging. Moreover; clinical staging was one of the independent relevant factors for prognosis in multivariate analysis.

Surgery is standard treatment for localized melanoma. There is no standard therapy for advanced-stage melanoma. In China the indications for treatment not only depend on the Chinese Guidelines for the Diagnosis and Treatment of Melanoma but also on patients' willingness and economic status. In our study, 68.7% of the patients underwent surgery. The 5-year survival rate of patients who had undergone surgery was significantly higher than that of the other patients. However, Cox multivariate analysis did not show it to be an independent relevant factor.

However, the present study still had several limitations. First, in China the indications for treatment depend not only on doctors' preferences but also on patients' willingness and economic status. These factors might have influenced the relatively poor survival result observed. Secondly, the sample was relatively small.

In conclusion, the presence of KLF6 in CMM was correlated with ulceration, lymph node metastasis and clinical stage. The 5-year survival rate in CMM patients was significantly associated with KLF6 expression, ulceration, lymph node metastasis, clinical stage and clinical operation. Ulceration and clinical staging were independent relevant factors for poor prognosis.

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