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Polo-Like Kinase 4 Correlates with Aggressive Tumor Characteristics, Shortened Disease-Free Survival and Overall Survival in Patients with Cutaneous Melanoma who Undergo Surgical Resection

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Polo-like kinase 4 (PLK4) involves in tumor progression via regulating centriole duplication. This study aimed to investigate correlations of PLK4 with tumor characteristics and survival in cutaneous melanoma patients undergoing surgical resection. Tumor specimens of 43 patients were retrieved for PLK4 determination by immunohistochemistry (IHC). The IHC score was a multiplication of staining intensity and percentage of staining-positive cells. This study found the median and mean tumor PLK4 IHC score was 0.0 (interquartile range: 0.0-6.0) and 3.5 ± 3.2 (mean ± SD), respectively. Elevated tumor PLK4 IHC score correlated with lymph node metastasis (P = 0.028), higher tumor node metastasis (TNM) stage (P = 0.004), and adjuvant therapy (P =0.029). Tumor PLK4 IHC score > 0 did not relate to disease-free survival (DFS) or overall survival (OS) (both P > 0.050). Tumor PLK4 IHC score > 3 associated with decreased DFS (P = 0.027), but not OS (P = 0.098). Five-year DFS rate of patients with tumor PLK4 IHC score = 0 and > 0 was 75.0% and 53.9%, correspondingly; while the rate of patients with the score ≤ 3 and > 3 was 81.0% and 37.5%, respectively. Five-year OS rate of patients with the score = 0 and > 0 was 100.0% and 66.3%, accordingly; whereas the rate of patients with the score ≤ 3 and > 3 was 85.7% and 61.5%, correspondingly. According to forward-step multivariate analysis, neither the score > 0 nor > 3 independently related to worse DFS and OS (all P > 0.050). Further validation via THE HUMAN PROTEIN ATLAS database showed high PLK4 RNA expression associated with shortened OS in melanoma patients (P = 0.001). PLK4 correlates with lymph node metastasis, increased TNM stage, and poor DFS in cutaneous melanoma patients undergoing surgical resection.

Keywords: cutaneous melanoma; polo-like kinase 4; survival; THE HUMAN PROTEIN ATLAS database; tumor features
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Introduction

Cutaneous melanoma is a malignancy caused by abnormal proliferation and uncontrolled division of skin melanocytes, with an increased incidence globally, especially in the white population (Arnold et al. 2022; Long et al. 2023). Ultraviolet radiation, associated with DNA damage and base mutation, is the most common risk factor for cutaneous melanoma (Schadendorf et al. 2018). Cutaneous melanoma can be classified into various types, such as superficial spreading, lentigomaligna, nodular, and others (Schadendorf et al. 2018; Long et al. 2023). The "ugly duckling" concept and the ABCD rule (asymmetry, border irregularities, color heterogeneity, dynamics, and evolution) combined with dermoscopy, are used for early clinical diagnosis of cutaneous melanoma (Michielin et al. 2019; Ahmed et al. 2020). The typical and preferred therapy is surgical resection for most local/locoregional and single distant

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metastasis cutaneous melanoma (Michielin et al. 2019). Nevertheless, post-surgical recurrence occurs to some cutaneous melanoma patients, with the rate varying from 18% to 55%, leading to worse prognosis (Statius Muller et al. 2002; Bamboat et al. 2014; Tarhini et al. 2018; Jang et al. 2020). Thus, finding biomarkers correlated with tumor characteristics and prognosis is required for cutaneous melanoma management.

Polo-like kinase 4 (PLK4), a serine/threonine protein kinase, plays a critical role in regulating cell cycle progression, especially in the control of centriole duplication (Zitouni et al. 2014). The deregulation of PLK4 can cause abnormal centrosome, mitosis, and chromosome, which are associated with tumorigenesis (Maniswami et al. 2018; Zhao and Wang 2019). Several studies have shown that PLK4 high expression is related with poor prognosis in some cancers (Duan et al. 2022; Jiang et al. 2022; Jiawei et al. 2022). For instance, one study indicates that PLK4 high expression reflects advanced tumor node metastasis (TNM) stage and short overall survival (OS) in patients with colorectal cancer (Duan et al. 2022). Another study elucidates that increased PLK4 expression is linked with poor relapse-free survival rates in breast cancer patients (Jiawei et al. 2022). In cutaneous melanoma, only one study states that PLK4 expression at the centrosomes of melanoma cancer is significantly elevated compared to benign nevi (Denu et al. 2018). However, the specific clinical role of PLK4 in patients with cutaneous melanoma remains unclear.

Hence, this study investigated the correlation of tumor PLK4 protein expression with tumor characteristics and survival in patients with cutaneous melanoma who underwent surgical resection.

Methods

Patients

The retrospective study included 43 patients with cutaneous melanoma who underwent surgical resection between January 2018 and May 2023. The inclusion criteria were: i) pathologically diagnosed as cutaneous melanoma; ii) aged ≥18 years; iii) underwent surgical resection; iv) had available records of follow-up; v) had available tumor specimens for the immunohistochemistry (IHC) assay of PLK4. The exclusion criteria were: i) had distant metastasis; ii) had severe cardiac, hepatic, or renal dysfunction; iii) had other malignant diseases. This study was endorsed by the Ethics Committee of Affiliated Hospital of Hebei Engineering University. Patients or their families, if the subjects had lost capacity to communicate or passed away at recruitment, submitted written informed consent.

Data sources and tumor specimens

The electronic records of patients were extracted from the hospital's electronic case system, from which data on clinical characteristics were obtained. Meanwhile, tumor specimens in the hospital were obtained.

IHC

IHC was used to detect PLK4 levels in the tumor specimens. Two antibodies were used during the experiment. PLK4 polyclonal antibody (Product No. PA5-29373; Thermo Fisher Scientific, Waltham, MA, USA) was used at a dilution of 1:1,000, and rabbit anti-human IgG1 recombinant secondary antibody (Product No. SA5-10202, Thermo Fisher Scientific) was used at a dilution of 1:15,000. The process was performed by an uninformed professional laboratory worker.

The results of IHC for PLK4 could be quantitatively assessed by the staining intensity and percentage of staining-positive cells, in which the intensity of staining was classified into four grades, namely 0 (none), 1+ (weak), 2+ (moderate), and 3+ (strong), while the percentage of staining-positive cells was classified into four grades, namely 1 (1-25%), 2 (26-50%), 3 (51-75%) and 4 (76-100%) (Chen et al. 2018). The IHC score was a multiplication of staining intensity and percentage of staining-positive cells, with a total score ranging from 0 to 12. The higher scores indicated higher levels of PLK4. The process was evaluated by two uninformed professional experimenters, and if the scores were different, a third experimenter was invited to score the IHC. The IHC score 0 and 3 was used as the cut-off value. In detail, the IHC score 0 was used to determine tumor PLK4 expression (yes vs. no) and the score 3 to determine tumor PLK4 expression (low vs. high). The cut-off values of 0 and 3 were used to analyze the association between tumor PLK4 IHC score and prognosis.

Evaluations

Follow-up information was obtained from the patient's electronic records. The final follow-up date was July 2023. The median follow-up was 29.5 months (range was 2.1-62.6 months). Disease-free survival (DFS) and overall survival (OS) were subsequently assessed based on the data.

Database assessment

This study extracted clinical characteristics of melanoma patients and their PLK4 RNA expression from THE HUMAN PROTEIN ATLAS database (https://www.proteinatlas.org). PLK4 RNA expression ≤ 2.340 was defined as low PLK4 RNA expression and its RNA expression > 2.340 was defined as high PLK4 RNA expression. Based on this database, the linkage of low and high PLK4 RNA expression with the clinical characteristics was analyzed. The comparison of prognosis between melanoma patients with low and high PLK4 RNA expression was also analyzed based on this database.

Data analysis

SPSS 22.0 (IBM Corp., Armonk, NY, USA) was used for data analysis in this study. For data presentation, continuous variables took the form of mean ± standard deviation (SD) or median and interquartile range (IQR), and categorical variables took the form of numbers (percentages).

Mann-Whitney U and Kruskal-Wallis H tests were used for comparative analysis. Spearman's test was used for correlation analysis. The Kaplan-Meier curves showed DFS or OS in patients with different Tumor PLK4 IHC scores (cut-off values of 0 and 3) by applying the log-rank test. Univariate and forward-step multivariate Cox proportional hazards regression analyses were used for evaluating factors associated with DFS and OS. *P*-values less than 0.05 were considered significant.

Results

Clinical features

The mean age of patients with cutaneous melanoma who underwent surgical resection was 60.6 ± 8.9 years, and 23 (53.5%) patients were ≥ 60 years old. Twenty-four (55.8%) patients were male. There were 20 (46.5%) patients with superficial spreading, 10 (23.3%) patients with lentigomaligna, 5 (11.6%) patients with nodular, and 8 (18.6%) patients with other or unspecified subtypes. Furthermore, 17 (39.5%) patients had lymph node metastasis, while no distant metastasis was observed. A total of 16 (37.2%), 10 (23.3%), and 17 (39.5%) patients were assessed as TNM stage I, II, and III, respectively. Regarding adjuvant therapy, 13 (30.2%) patients received interferon alpha, 8 (18.6%) patients received programmed death 1 inhibitor, and 2 (4.7%) patients received vemurafenib. The detailed clinical features were listed in Table 1.

Tumor PLK4 IHC score

The examples of tumor PLK4 IHC score = 0, 1-3, and > 3 were presented in Fig. 1. In patients with cutaneous melanoma who underwent surgical resection, there were 11 (25.6%) patients with tumor PLK4 IHC score = 0, 12 (27.9%) patients with tumor PLK4 IHC score 1-3, and 20 (46.5%) patients with tumor PLK4 IHC score > 3. The minimum and the maximum of tumor PLK4 IHC score was 0.0 and 12.0, accordingly. The median (IQR) tumor PLK4 IHC score was 3.0 (0.0-6.0). The mean tumor PLK4 IHC score was 3.5 ± 3.2 (mean ± SD).

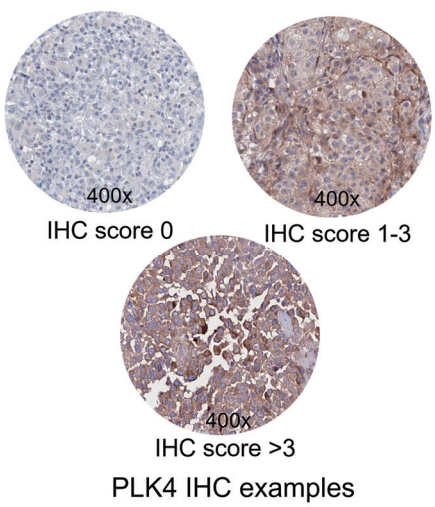
Correlation of tumor PLK4 IHC score with clinical features

In patients with cutaneous melanoma who underwent surgical resection, elevated tumor PLK4 IHC score was related to lymph node metastasis ($P = 0.028$). Tumor PLK4 IHC score was positively correlated with TNM stage in these patients ($P = 0.004$). Increased tumor PLK4 IHC score was associated with adjuvant therapy in these patients

Table 1. Clinical characteristics.

Items	Cutaneous melanoma patients (N = 43)
Age (years), mean ± SD	60.6 ± 8.9
Age ≥ 60 years, n (%)	23 (53.5)
Male, n (%)	24 (55.8)
Subtype, n (%)	
Superficial spreading	20 (46.5)
Lentigomaligna	10 (23.3)
Nodular	5 (11.6)
Others or unspecified	8 (18.6)
Tumor diameter > 2 cm, n (%)	25 (58.1)
Lymph node metastasis, n (%)	17 (39.5)
Distant metastasis, n (%)	0 (0.0)
TNM stage, n (%)	
I	16 (37.2)
II	10 (23.3)
III	17 (39.5)
Adjuvant therapy, n (%)	23 (53.5)
IFN-α	13 (30.2)
PD-1 inhibitor	8 (18.6)
Vemurafenib	2 (4.7)

SD, standard deviation; TNM, tumor node metastasis; IFN-α, interferon alpha; PD-1, programmed death 1.



IHC score	Cutaneous melanoma patients (N = 43)
=0, n (%)	11 (25.6)
1-3, n (%)	12 (27.9)
>3, n (%)	20 (46.5)
Minimum	0.0
Quartile 1	0.0
Median	3.0
Quartile 3	6.0
Maximum	12.0
Mean	3.5
SD	3.2

Fig. 1. Representations of tumor PLK4 IHC score examples and distribution of tumor PLK4 IHC score. PLK4, Polo-like kinase 4; IHC, immunohistochemistry; SD, standard deviation.

(P = 0.029). Additionally, no association was found in tumor PLK4 IHC score with age ≥ 60 years (P = 0.155), sex (P = 0.673), tumor subtype (P = 0.934), or tumor diameter > 2 cm (P = 0.056) (Table 2).

Correlation of tumor PLK4 IHC score with DFS and OS

A total of 9 (20.9%) patients had disease progression, and 5 (11.6%) patients died. In patients with cutaneous melanoma who underwent surgical resection, tumor PLK4 IHC score > 0 was not related to DFS (P = 0.212). The 1-year, 3-year, and 5-year accumulating DFS rate of patients with tumor PLK4 IHC score = 0 was 100.0%, 100.0%, and 75.0%, respectively; while the 1-year, 3-year, and 5-year accumulating DFS rate of patients with tumor PLK4 IHC score > 0 was 89.3%, 67.3%, and 53.9%, accordingly (Fig. 2A). Tumor PLK4 IHC score > 3 was correlated with shortened DFS (P = 0.027). Detailly, the 1-year, 3-year, and 5-year accumulating DFS rate of patients with tumor PLK4 IHC score ≤ 3 was 100.0%, 94.4%, and 81.0%, correspondingly; while the 1-year, 3-year, and 5-year accumulating DFS rate of patients with tumor PLK4 IHC score > 3 was 82.8%, 56.2%, and 37.5%, separately (Fig. 2B).

Tumor PLK4 IHC score > 0 was not associated with OS (P = 0.180). The 1-year, 3-year, and 5-year accumulating OS rate of patients with tumor PLK4 IHC score = 0 was all 100.0%; while that rate of patients with tumor PLK4 IHC score > 0 was 100.0%, 85.2%, and 66.3%, respectively (Fig. 2C). Tumor PLK4 IHC score > 3 was not related to OS (P = 0.098), either. In detail, the 1-year, 3-year, and 5-year accumulating OS rate of patients with tumor PLK4 IHC score ≤ 3 was 100.0%, 100.0%, and 85.7%, correspondingly; while that rate of patients with tumor PLK4 IHC score > 3 was 100.0%, 76.9%, and 61.5%, accordingly (Fig. 2D).

Tumor PLK4 IHC score > 6 was associated with shortened DFS (P = 0.014). The 1-year, 3-year, and 5-year accumulating DFS rate of patients with tumor PLK4 IHC score ≤ 6 was 96.6%, 83.6%, 75.2%, accordingly; while that rate of patients with tumor PLK4 IHC score > 6 was 75.0%, 37.5%, and 0.0%, correspondingly (Supplementary Fig. S1).

Related factors of DFS and OS

In patients with cutaneous melanoma who underwent surgical resection, tumor PLK4 IHC score > 3 [hazard ratio (HR): 4.978, P = 0.046], lymph node metastasis (HR: 5.124, P = 0.024), and higher TNM stage (HR: 3.061, P = 0.015) were correlated with worse DFS, while tumor PLK4 IHC score > 0 was not correlated with DFS (P = 0.242). After

Table 2. Correlation of PLK4 IHC score with clinical characteristics of patients.

Items	PLK4 IHC score, median (IQR)	r/Z/X^2 value	P value
Age ≥ 60 years		−1.421	0.155
No	2.5 (0.0-4.0)		
Yes	4.0 (2.0-8.0)		
Sex		−0.422	0.673
Female	3.0 (1.0-4.0)		
Male	4.0 (0.0-7.5)		
Subtype		0.431	0.934
Superficial spreading	2.0 (0.0-7.5)		
Lentigomaligna	3.5 (1.5-6.0)		
Nodular	4.0 (0.0-7.0)		
Others or unspecified	4.0 (2.0-5.5)		
Tumor diameter > 2 cm		−1.912	0.056
No	2.0 (0.0-4.5)		
Yes	4.0 (2.0-7.0)		
Lymph node metastasis		−2.194	0.028
No	2.0 (0.0-4.5)		
Yes	4.0 (2.0-8.0)		
TNM stage		0.426	0.004
I	1.5 (0.0-3.0)		
II	3.5 (1.8-6.0)		
III	4.0 (2.0-8.0)		
Adjuvant therapy		−2.188	0.029
No	2.0 (0.0-3.8)		
Yes	4.0 (2.0-8.0)		

PLK4, polo-like kinase 4; IHC, immunohistochemistry; IQR, interquartile range; TNM, tumor node metastasis.

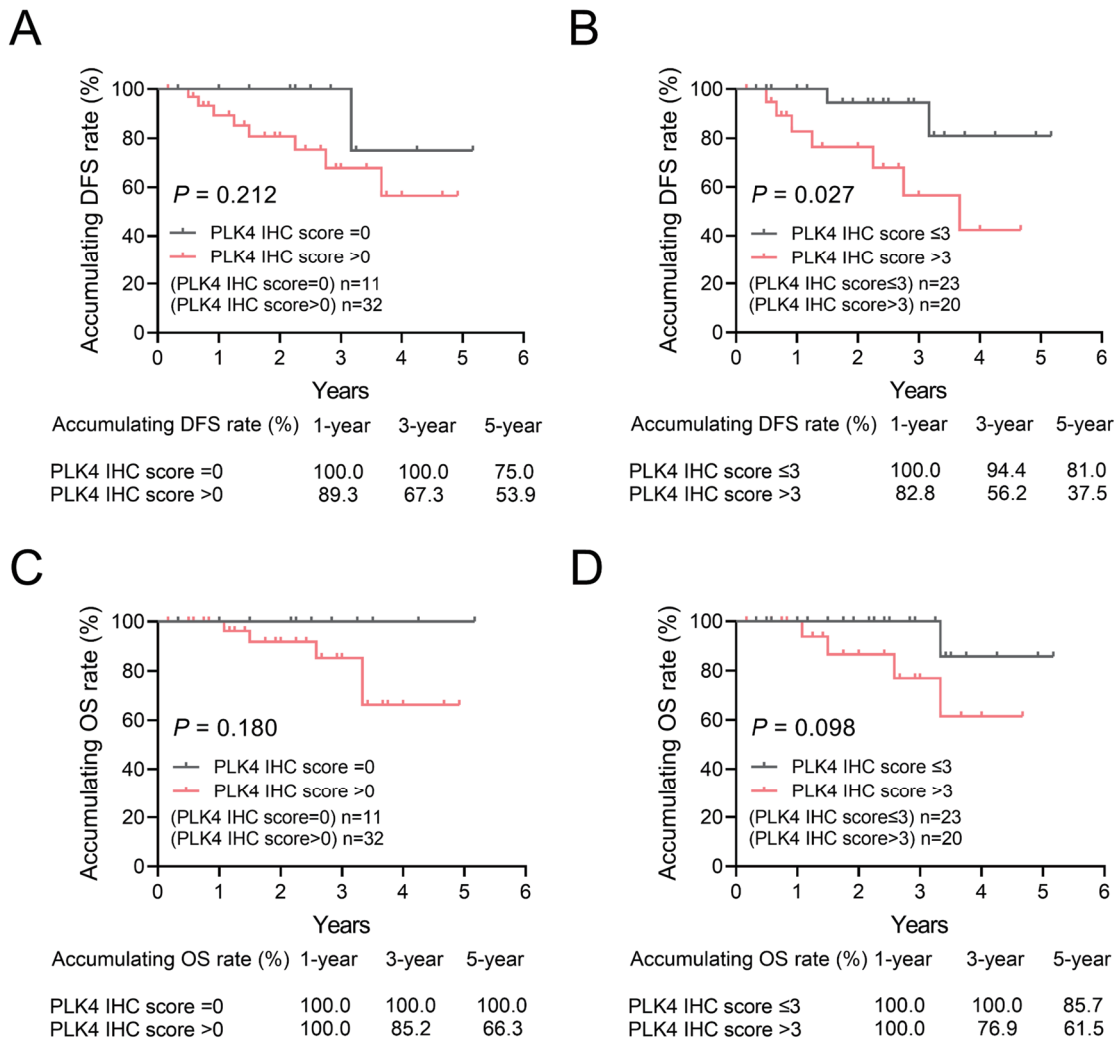


Fig. 2. Correlation of tumor PLK4 IHC score with disease-free survival (DFS) and overall survival (OS). Tumor PLK4 IHC score > 3 was correlated with shortened DFS in patients with cutaneous melanoma who underwent surgical resection. (A and B) Correlation of DFS with tumor PLK4 IHC score > 0 (A) and > 3 (B) in patients with cutaneous melanoma who underwent surgical resection. (C and D) Correlation of OS with tumor PLK4 IHC score > 0 (C) and > 3 (D) in patients with cutaneous melanoma who underwent surgical resection.
PLK4, Polo-like kinase 4; IHC, immunohistochemistry.

forward-step multivariate analysis, higher TNM stage (HR: 3.061, $P = 0.015$) was independently associated with shortened DFS in these patients (Table 3).

Tumor PLK4 IHC score > 3 ($P = 0.145$) and tumor PLK4 IHC score > 0 ($P = 0.425$) were not related to OS in these patients, and other clinical features were not correlated with OS, either (all $P > 0.050$). After adjustment, no factors were independently linked with OS in these patients (Table 4).

Public database validation

According to THE HUMAN PROTEIN ATLAS database, there was no correlation of PLK4 RNA expression with age ($P = 0.716$), sex ($P = 0.137$), race ($P = 0.425$), or TNM stage ($P = 0.667$) in melanoma patients (Table 5). Concerning the survival, PLK4 RNA expression was negatively correlated with OS in melanoma patients ($P = 0.001$). The median [95% confidence interval (CI)] OS in patients with low PLK4 RNA expression (determined by the median) was 3.7 (1.8-5.6) years, while the median (95% CI) OS in patients with high PLK4 RNA expression was 1.7 (1.0-2.4) years. The 1-year, 3-year, and 5-year accumulating OS rate of patients with low PLK4 RNA expression was 92.9%, 55.0%, and 27.5%, respectively; while 1-year, 3-year, and 5-year accumulating OS rate of patients with high PLK4 RNA expression was 80.9%, 0.0%, and 0.0%, correspondingly (Table 6).

Discussion

Previous studies have revealed that the correlation of PLK4 with tumor features (Duan et al. 2022; Jiang et al. 2022; Jiawei et al. 2022). For example, one study indicates

Table 3. Cox proportional hazards regression analyses for disease-free survival (DFS).

Items	*P* value	HR	95% CI for HR	
			Lower	Upper
Univariate analysis				
PLK4 IHC score > 3, yes vs. no	0.046	4.978	1.031	24.040
PLK4 IHC score > 0, yes vs. no	0.242	3.468	0.433	27.795
Age ≥ 60 years, yes vs. no	0.218	2.691	0.558	12.984
Sex, male vs. female	0.563	1.509	0.374	6.084
Subtype				
Superficial spreading	Reference			
Lentigomaligna	0.768	0.780	0.150	4.057
Nodular	0.638	1.702	0.186	15.588
Others or unspecified	0.522	0.495	0.058	4.263
Tumor diameter > 2 cm, yes vs. no	0.069	4.412	0.892	21.833
Lymph node metastasis, yes vs. no	0.024	5.124	1.241	21.149
Higher TNM stage	0.015	3.061	1.238	7.567
Adjuvant therapy, yes vs. no	0.175	2.657	0.646	10.920
Forward-step multivariate analysis				
Higher TNM stage	0.015	3.061	1.238	7.567

HR, hazard ratio; CI, confidence interval; PLK4, polo-like kinase 4; IHC, immunohistochemistry; TNM, tumor node metastasis.

Table 4. Cox proportional hazards regression analyses for overall survival (OS).

Items	*P* value	HR	95% CI for HR	
			Lower	Upper
Univariate analysis				
PLK4 IHC score > 3, yes vs. no	0.145	5.117	0.571	45.844
PLK4 IHC score > 0, yes vs. no	0.425	32.057	0.006	1.607×10^5
Age ≥ 60 years, yes vs. no	0.399	2.575	0.286	23.187
Sex, male vs. female	0.296	3.225	0.358	29.037
Subtype				
Superficial spreading	Reference			
Lentigomaligna	0.966	1.053	0.095	11.622
Nodular	0.228	4.880	0.371	64.250
Others or unspecified	0.903	1.161	0.105	12.882
Tumor diameter > 2 cm, yes vs. no	0.183	4.461	0.494	40.240
Lymph node metastasis, yes vs. no	0.216	151.327	0.053	4.332×10^5
Higher TNM stage	0.194	17.816	0.232	1.369×10^3
Adjuvant therapy, yes vs. no	0.241	76.011	0.055	1.055×10^5
Forward-step multivariate analysis				
Lymph node metastasis, yes vs. no	0.216	151.327	0.053	4.332×10^5

HR, hazard ratio; CI, confidence interval; PLK4, polo-like kinase 4; IHC, immunohistochemistry; TNM, tumor node metastasis.

that tumor PLK4 expression is associated with advanced TNM stage in colorectal cancer patients (Duan et al. 2022). Another study elucidates that upregulation of PLK4 expression is linked with poor tumor differentiation and increased TNM stage in renal cell carcinoma patients (Jiang et al. 2022). This study also found that elevated tumor PLK4 IHC score was correlated with lymph node metastasis, higher TNM stage, and adjuvant therapy in patients with cutaneous melanoma who underwent surgical resection. The possible explanation could be: (1) PLK4 promoted cutaneous melanoma cell metastasis via regulating the actin cytoskeleton (Kazazian et al. 2017). Consequently, PLK4

Table 5. PLK4 and clinical characteristics of melanoma patients in THE HUMAN PROTEIN ATLAS database.

Items	n, (%)	PLK4 expression[a]			
		Low, n (%)	High, n (%)	*r/Z* value	*P* value
Age (years)				−0.364	0.716
< 60	38 (37.3)	25 (65.8)	13 (34.2)		
≥ 60	64 (62.7)	40 (62.5)	24 (37.5)		
Sex				−1.486	0.137
Female	42 (41.2)	31 (73.8)	11 (26.2)		
Male	60 (58.8)	34 (56.7)	26 (43.3)		
Race				−0.798	0.425
Asian	7 (6.9)	4 (57.1)	3 (42.9)		
White	93 (91.1)	59 (63.4)	34 (36.6)		
Unknown	2 (2.0)	2 (100.0)	0 (0.0)		
TNM stage				−0.044	0.667
I	2 (2.0)	1 (50.0)	1 (50.0)		
II	65 (63.7)	40 (61.5)	25 (38.5)		
III	27 (26.5)	20 (74.1)	7 (25.9)		
IV	3 (2.9)	0 (0.0)	3 (100.0)		
Unknown	5 (4.9)	4 (80.0)	1 (20.0)		

[a]PLK4 expression ≤ 2.340 was defined as the low PLK4 expression, and PLK4 expression > 2.340 was defined as the high PLK4 expression.
PLK4, polo-like kinase 4.

Table 6. Correlation of PLK4 with the accumulating overall survival (OS) rate based on THE HUMAN PROTEIN ATLAS database.

Items	Low PLK4 expression[a] (N = 65)	High PLK4 expression[b] (N = 37)
P value by Log-rank test[c]	0.001	
Median OS (years)	3.7	1.7
95% CI for OS	1.8-5.6	1.0-2.4
1-year accumulating OS rate (%)	92.9	80.9
3-year accumulating OS rate (%)	55.0	0.0
5-year accumulating OS rate (%)	27.5	0.0

[a]PLK4 expression ≤ 2.340 was defined as the low PLK4 expression.
[b]PLK4 expression > 2.340 was defined as the high PLK4 expression.
[c]Comparison of the accumulating OS rate between high and low expression of PLK4.
PLK4, polo-like kinase 4; CI, confidence interval.

was positively associated with lymph node metastasis in patients with cutaneous melanoma who underwent surgical resection. (2) Elevated PLK4 was implicated in the growth and progression of cutaneous melanoma through excessive centrosome amplification (Levine et al. 2017; Hoffmann 2022). Thus, PLK4 was positively related to TNM stage in patients with cutaneous melanoma who underwent surgical resection. (3) Adjuvant therapy was recommended for patients who were at high risk or those with metastasis, typically at stage IIB-III, meanwhile, PLK4 was correlated with poor tumor features discussed above (Michielin et al. 2019; Bruce et al. 2021). Thereby, PLK4 was positively linked with adjuvant therapy in patients with cutaneous melanoma who underwent surgical resection. However, most of these explanations were hypotheses based on previous studies, therefore, further explorations were required to verify these hypotheses in cutaneous melanoma.

It is elucidated that high PLK4 expression is associated with poor survival in patients with different cancers (Zeng et al. 2020; Hu et al. 2022; Zhang et al. 2022). For instance, one study demonstrates that high PLK4 expression is correlated with shortened OS in patients with clear cell renal cell carcinoma (Hu et al. 2022). Another study indicates that PLK4 is independently correlated with poor DFS and OS in patients with non-small cell lung cancer (Zeng et al. 2020). This study found that tumor PLK4 had a certain predictive effect on DFS in patients with cutaneous melanoma who underwent surgical resection, and tumor PLK4 IHC score with a cut-off value of 3 suggested a better ability of predicting DFS than that score with a cut-off value of 0. The possible explanations could be: (1) As found in this study, PLK4 was positively correlated with

lymph node metastasis and TNM stage, which could result in poor clinical outcomes. Thus, PLK4 was negatively related to DFS in patients with cutaneous melanoma who underwent surgical resection (Buja et al. 2021; Subramanian et al. 2021). (2) Tumor PLK4 IHC score > 0 showed a correlating trend with shortened DFS, but the number of patients with tumor PLK4 IHC score = 0 was relatively small, resulting in a great influence of censored data and progression cases on the results. Thereby, the cut-off value of 0 did not show obvious predicting capacity of survival in patients with cutaneous melanoma who underwent surgical resection.

Moreover, this study showed that tumor PLK4 IHC score was not linked with OS, which could be explained by the fact that the incidence of cutaneous melanoma is relatively low in China (less than 1 per 100,000 person-years, with an incidence rate of 2.4% in 2020) (Arnold et al. 2022). Despite the effort to enroll as many as possible eligible patients, the sample size was still small in this study. Therefore, the correlation of PLK4 with OS was not obvious in these patients. No factor was found to be linked with OS in this study, which might due to the relatively small number of endpoint events, with 5 (11.6%) patients experiencing mortality.

Furthermore, this study utilized THE HUMAN PROTEIN ATLAS database for verification and the findings suggested that PLK4 RNA expression was negatively correlated with OS in melanoma patients, which was different from the findings of this study. The possible explanations could be: (1) There were 65 (63.7%) patients with low PLK4 RNA expression and 37 (36.3%) patients with high PLK4 RNA expression according to the public database. Whereas in this study, 12 (27.9%) patients had low tumor PLK4 expression and 20 (46.5%) patients had high tumor PLK4 expression, indicating that PLK4 might have a predictive value for OS, and the relatively small sample size of this study weakened the statistical power. (2) This study detected tumor PLK4 protein expression, while THE HUMAN PROTEIN ATLAS database determined PLK4 RNA expression, suggesting that the association between PLK4 and OS of melanoma patients might be attributed to nucleic acid-based mechanisms. Consequently, the findings of this study were different from that of the public database. (3) High PLK4 expression reflected the strong ability of proliferation and metastasis in melanoma cells, which led to poor survival (Jiang et al. 2022). Thereby, PLK4 RNA expression was negatively correlated with OS in melanoma patients. Besides, the survival of patients in this study were better than that in THE HUMAN PROTEIN ATLAS database. The possible explanation could be that all patients in this study received surgical resection without distant metastasis, but THE HUMAN PROTEIN ATLAS did not exclude patients with distant metastasis whose survival was worse.

There were some limitations in this study. Firstly, this study was a retrospective study, which could exist selective bias. Secondly, further studies were required to explore whether PLK4 could be targeted for the treatment of cutaneous melanoma. Finally, since this study did not enroll cutaneous melanoma patients at TNM stage IV, the findings of this study might not be applicable in these patients.

To sum up, elevated tumor PLK4 expression reflects lymph node metastasis, higher TNM stage, and shortened DFS in patients with cutaneous melanoma who undergo surgical resection. The findings provide some evidences for clinicians to execute timely intervention in these patients with worse survival.

Conflict of Interest

The authors declare no conflict of interest.

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Supplementary Files

Please find supplementary file(s);
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