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Citation: Shi X, Zhou Q, Huang B, Xia S, Jiang Y, Fang S, et al. (2022) Prognostic and immunerelated value of *STK17B* in skin cutaneous melanoma. PLoS ONE 17(2): e0263311. https:// doi.org/10.1371/journal.pone.0263311

Editor: Salvatore V Pizzo, Duke University School of Medicine, UNITED STATES

Received: August 17, 2021

Accepted: January 16, 2022

Published: February 16, 2022

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Data Availability Statement: All relevant data are within the manuscript and its Supporting information files.

Funding: This research was funded by the National Natural Science Foundation of China (81973603), Natural Science Foundation of Liaoning Province (No.2019-MS-094), Authors declared that the funding source did not participate in the study design; collection, analyses and interpretation of data; in the writing of the manuscript; and in the decision to submit the manuscript for publication.

RESEARCH ARTICLE

Prognostic and immune-related value of *STK17B* in skin cutaneous melanoma

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Abstract

Skin cutaneous melanoma (SKCM) is a common cancer of which mortality is increasing continuously. Our study conducted a series of analyses on the clinical significance of Serine/threonine kinase 17B (STK17B) in SKCM to provide a new biomarker for diagnosis and treatment. The RNA-sequence data were obtained from The Cancer Genome Atlas and Genotype-Tissue Expression databases. The data of 468 SKCM patients were divided into STK17B high- and low-expression groups and analyzed by Bioconductor package to identify the differential expressed genes. The R package of "clusterProfiler" was used for Gene Ontology, Kyoto Encyclopedia of Genes and Genomes, and Gene-Set Enrichment Analysis analyses. A protein-protein interaction network and immune infiltration landscape were respectively constructed via STRING database and ssGSEA. STK17B had lower expression in SKCM than normal tissues. Besides, STK17B expression was significantly related to some clinicopathological characteristics in SKCM patients including T stage, Breslow depth, radiation therapy, melanoma Clark level, and pathologic stage. The Kaplan-Meier curve analyses revealed that the low expression of STK17B was correlated with poor overall survival and disease-specific survival. We constructed nomograms to predict the 1-, 3-, and 5year survival of SKCM patients. The function enrichment analyses showed STK17B-related differential expressed genes were enriched in cellular differentiation and immune-related progress. STK17B expression level were positively correlated with infiltrating level of immune cells. In this study, we found that STK17B, which played an important role in immune infiltration, could be a new biomarker for diagnosis and prognosis in SKCM patients.

Competing interests: We declare that they have no conflicts of Interest.

1. Introduction

Skin cutaneous melanoma (SKCM) is one of the most malignant skin tumors, which can both locally invade surrounding tissues as well as metastasize systemically. The global incidence of SKCM continues to increase and it is particularly common in fair-skinned populations [1]. The morbidity of SKCM is only 4% to 11% of all skin cancers but the mortality of SKCM is close to three quarters of total fatality rate from skin tumors [2]. As is known, a main risk factor of melanoma is ultraviolet radiation. However, SKCM can also appear in non-sun-exposed area, such as feet, mouth, and nasal passages, caused by dysplastic naevi or hereditary factors [3]. SKCM can be cured through surgical excision if diagnosed in early stage. Immunotherapy and targeted therapies are also used clinically. However, untreated stage IV patients survive less than one year, it is urgent to find novel and effective targets for clinical research and therapeutic method [4].

Serine/threonine kinase 17B (STK17B), also known as DAP kinase-related apoptosis-inducing protein kinase 2 (DRAK2), is located on chromosome 2 (2q32.3). As a member of DAPK family, that all members have been reported inducing apoptosis through abnormal expression in various cell types, STK17B is still in dispute for its effect on apoptosis. STK17B was also a negative regulator of TGF- β signaling, which is known as a crucial step in the tumorigenic development, inhibiting the phosphorylation of R-Smads through its interaction with T β RI. Additionally, STK17B was over-expressed in basal-like and HER2-enriched breast cancer, and silence of STK17B retarded tumorigenesis and tumor growth in xenograft model [5]. STK17B is upregulated in hepatocellular carcinoma tissues and cell lines. At the same time, this gene has been identified facilitating carcinogenesis and metastasis [6]. Besides, there is few literature about the relationship of STK17B and melanoma.

In this study, we indicated the clinical diagnosis and prognostic value of STK17B in SKCM. Firstly, we downloaded the RNA-Seq data from The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) database, and explored a correlation between STK17B and SKCM. A contrastive analysis of STK17B differential expression was performed between SKCM and normal tissues. The diagnostic and prognostic value of this gene were estimated and nomograms were drawn to predict the 1-, 3-, 5-year overall survival (OS), disease-specific survival (DSS), and progression-free interval (PFI) of SKCM patients. The immune infiltration landscape of STK17B was quantified by single-sample gene set enrichment analysis (ssGSEA) method. Furthermore, the SKCM patient samples were divided into high- and low-expression groups according to the expression level of STK17B. The STK17B-related differential expressed genes (DEGs) were identified by comparing the sequencing data of the two groups. Subsequently, the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses was performed for STK17B-related DEGs by R package. Gene set enrichment analysis (GSEA) to filtrate significantly enriched gene sets. In addition, we constructed a protein-protein interaction (PPI) network of STK17B. The present study showed that lower STK17B expression was related to poor prognosis and can be considered as a potential diagnostic and prognostic biomarker of SKCM.

2. Materials and methods

2.1. RNA-sequencing patient data and expression analysis

The RNA-Seq data of TCGA and GTEx database were downloaded from the University of California Santa Cruz (UCSC) XENA (https://xenabrowser.net/datapages/), which were handled by the Toil process and transformed into transcripts per million reads [7]. The expression of STK17B in various types of cancers was analyzed. Patients with unavailable clinical information were excluded. In addition, total of 1282 normal and SKCM samples with detailed clinicopathological information were collected and further analyzed.

2.2. A receiver operating characteristic (ROC) analysis and survival analysis

A receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic effectivity for SKCM, and the area under the curve was calculated to assess value of the predictive value of the testing method. According to the median value of STK17B mRNA expression, patients were divided into STK17B high- and low-expression groups. Subsequently, the R package of "survminer" was used to assess the prognostic value of STK17B for the OS and DSS in SKCM. Furthermore, the prognostic value of STK17B was analyzed in subgroups of SKCM patients with different clinicopathologic characteristics, such as pathologic stage, radiation therapy, gender, age.

2.3. Construction of nomogram and forest plots

According to the results of Cox regression, nomogram models were constructed to calculate the predictive value of STK17B in estimating the prognosis of SKCM patients. Calibration plots were also performed to assess the effectiveness of the model. In addition, forest plots were constructed to visualize the prognostic value of STK17B for OS, DSS, and PFI in SKCM patients.

2.4. Analysis of immune infiltration

The TCGA gene expression dataset was used to quantify immune infiltration landscape of STK17B by ssGSEA method, and the marker genes of 24 immune cells types were previously reported [8]. The ssGSEA analysis was performed by GSVA package from R program (http:// www.bioconductor.org) [9]. The correlation between immune cells and STK17B was analyzed by Spearman's rank-correlation coefficient [10]. Infiltration levels of immune cells between STK17B high- and low-expression group was compared by Wilcoxon rank sum test [11]. The R package estimate was used to analyze the relationship of immune score and STK17B expression [12]. Six immune subtypes were identified according to different immune expression signatures of tumor, including C1 (wound healing), C2 (IFN- γ dominant), C3 (inflammatory), C4 (lymphocyte depleted), C5 (immunologically quiet), and C6 (TGF- β dominant) [13]. We also analyzed STK17B expression in different immune subtypes using TISIDB (http://cis.hku. hk/TISIDB/index.php) [14, 15].

2.5. Analysis of STK17B-related DEGs for SKCM between STK17B highand low-expression groups

The Bioconductor package "DESeq2" was used to compare the RNA-seq of STK17B high- and low-expression groups and identify STK17B-related DEGs in SKCM [16]. The adjusted P < 0.05 and $|\log_2$ Fold change (FC)| >2 were selected as cut-off criteria to identify STK17B-related DEGs. A volcano plot and heat map were drawn using ggplot2 packages of R for the visualization of the identified STK17B-related DEGs.

2.6. Functional enrichment analysis

The clusterProfiler package was used to perform GO and KEGG analysis of STK17B-related DEGs [17]. GO is a comprehensive source of digital data relating to the functions of genes in

three independent categories: molecular function, biological process, and cellular component. The significant P-value was adjusted by Benjamin and Hochberg method.

2.7. Gene set enrichment analysis (GSEA) and protein-protein interaction (PPI) network

GSEA is a computational method to identify significantly enriched or depleted groups of genes. GSEA was performed by R package of clusterProfiler based on the STK17B-related DEGs [17]. In this study, GSEA was used to identify significantly enriched gene sets between STK17B high- and low-expression groups. The gene sets with a nominal P < 0.05 and a false discovery rate < 0.05 were considered as significantly enrichment. We analyzed the PPI network of STK17B via the STRING database (https://string-db.org/), and the minimum required interaction cutoff is 0.4 [18]. Subsequently, the PPI network was constructed by Cytoscape software (3.8.0) [19].

3. Results

3.1. The expression levels of STK17B

We explored the expression differences of STK17B in 33 human cancers and corresponding normal tissues based on the TCGA and UCSC data sets. The association between STK17B expression in various types of cancers was analyzed (Fig 1A). Compared to normal samples, dramatically lower expression of STK17B was showed in SKCM (Fig 1B). Furthermore, we evaluated the diagnostic value of STK17B in SKCM patients by ROC curve analysis (Fig 1C). the area under the curve value of the ROC curve of STK17B was 0.734 (95% confidence interval [CI] = 0.701-0.768), suggesting that normal tissues could be effectively distinguished from SKCM tissues according to the expression level of STK17B.

3.2. Correlation between STK17B expression and clinicopathological characteristics in SKCM

The correlation between the STK17B expression and clinicopathological characteristics of SKCM patients was analyzed (Table 1). The correlation results indicated that STK17B expression was significantly associated with T stage, Breslow depth and Radiation therapy in SKCM patients (P = 0.013, P = 0.009, P < 0.001, respectively). However, the results showed that the STK17B expression were not significantly associated with other parameters, including N, M stage, pathologic stage, melanoma Clark level, melanoma ulceration, gender, race, tumor tissue site, BRAF status and age (all P > 0.05).

3.3. STK17B is an independent predictor of prognosis in SKCM

The effects of STK17B on the DSS and OS of SKCM patients were analyzed using the Kaplan-Meier curve (Fig 1D and 1E). The results showed that lower expression of STK17B was associated with worse OS (HR = 0.69(0.53–0.91), P = 0.008) and DSS (HR = 0.68(0.51–0.90), P = 0.008). In the univariate Cox regression analysis, T, N stage, pathologic stage, melanoma Clark level, Breslow depth, melanoma ulceration, age, race, STK17B were all associated with OS (Table 2). TNM stage, pathologic stage, melanoma Clark level, Breslow depth, melanoma ulceration, age, STK17B, were all associated with DSS (Table 3). The variables with P < 0.1 in the univariate analyses were included in the subsequent multivariate Cox regression analysis. Multivariate analyses demonstrated that N stage, Breslow depth, STK17B expression were independent prognostic factors in OS (P < 0.05) (Table 2). Similarly, N stage, Breslow depth, STK17B were independent prognostic factors in DSS (P < 0.05) (Table 3). Therefore, low



Fig 1. STK17B expression and prognosis in skin cutaneous melanoma (SKCM) patients. (A) The association between STK17B expression in various types of cancers; **(B)** STK17B has low expression in SKCM tissues; **(C)** the diagnostic value of STK17B in SKCM patients by receiver operating characteristic curve analysis; **(D, E)** the prognostic value of STK17B for disease-specific survival and overall survival in SKCM patients.

Characters	level	Low expression of STK17B	High expression of STK17B	Р
n		234	234	
T stage (%)	T1	16(8.6%)	25(14.4%)	0.013
	T2	39(20.9%)	39(22.4%)	
	T3	39(20.9%)	51(29.3%)	
	T4	93(49.7%)	59(33.9%)	
N stage (%)	N0	116(56.0%)	118(57.8%)	0.708
	N1	39(18.8%)	35(17.2%)	
	N2	22(10.6%)	27(13.2%)	
	N3	30(14.5%)	24(11.8%)	
M stage (%)	M0	210(94.6%)	206(94.5%)	1.000
	M1	12(5.4%)	12(5.5%)	
Pathologic stage (%)	Stage I	32(15.4%)	44(21.9%)	0.197
	Stage II	80(38.5%)	60(29.9%)	
	Stage III	85(40.9%)	85(42.3%)	
	Stage IV	11(5.3%)	12(6.0%)	
Melanoma Clark level (%)	I	4(2.5%)	2(1.2%)	0.057
	п	6(3.8%)	12(7.5%)	
	III	29(18.2%)	47(29.4%)	
	IV	91(57.2%)	77(48.1%)	
	V	29(18.2%)	22(13.8%)	
Breslow depth (%)	< = 3	80(44.4%)	104(58.8%)	0.009
	>3	100(55.6%)	73(41.2%)	
Melanoma ulceration (%)	No	70(42.4%)	75(51.4%)	0.143
	Yes	95(57.6%)	71(48.6%)	
Radiation therapy (%)	No	206(90.0%)	175(75.4%)	< 0.001
	Yes	23(10.0%)	57(24.6%)	
Gender (%)	Female	90(38.5%)	89(38.0%)	1.000
	Male	144(61.5%)	145(62.0%)	
Race (%)	Asian	9(4.0%)	3(1.3%)	0.086
	Black or African American	0(0.0%)	1(0.4%)	
	White	218(96.0%)	227(98.3%)	
Tumor tissue site (%)	Extremities	109(51.4%)	86(42.2%)	0.126
	Head and Neck	20(9.4%)	17(8.3%)	
	Other Specify	4(1.9%)	9(4.4%)	
	Trunk	79(37.3%)	92(45.1%)	
BRAF status (%)	Mutant	106(45.5%)	126(54.3%)	0.071
	Wild Type	127(54.5%)	106(45.7%)	
Age (median [IQR])		58.00[48.25,71.00]	60.00[46.25,70.00]	0.444

Table 1. The correlation between the STK17B expression and clinicopathological characteristics of skin cutaneous melanoma patients.

https://doi.org/10.1371/journal.pone.0263311.t001

STK17B expression was an independent risk associated with poor prognosis in SKCM patients.

3.4. The relationship of STK17B and prognosis of SKCM patients with different clinicopathological status

A further subgroup analysis showed that low STK17B was correlated with worse OS in N and M Stage, pathologic stage, radiation therapy, gender, race, melanoma ulceration, age,

Characteristics	Total (N)	HR (95% CI) Univariate analysis	P value Univariate analysis	HR (95% CI) Multivariate analysis	P value Multivariate analysis
T stage (T3&T4 vs. T1&T2)	358	2.040(1.468-2.836)	< 0.001	0.956(0.541-1.688)	0.876
N stage (N1&N2&N3 vs. N0)	399	1.711(1.271-2.304)	< 0.001	3.519(1.030-12.019)	0.045
M stage (M1 vs. M0)	427	1.734(0.915-3.287)	0.092	1.886(0.629-5.656)	0.258
Pathologic stage (Stage III &Stage IV vs. Stage I &Stage II)	407	1.579(1.177–2.118)	0.002	0.628(0.179–2.204)	0.468
Melanoma Clark level (IV&V vs. I&II&III)	312	2.117(1.472-3.045)	< 0.001	1.229(0.746-2.024)	0.419
Breslow depth (>3 vs. $< = 3$)	352	2.593(1.892-3.553)	< 0.001	1.757(1.026-3.010)	0.040
Melanoma ulceration (Yes vs. No)	310	2.087(1.494-2.916)	< 0.001	1.456(0.954-2.220)	0.081
Radiation therapy (Yes vs. No)	447	0.953(0.674-1.348)	0.785		
Age (>60 vs. < = 60)	453	1.678(1.266-2.225)	< 0.001	1.242(0.820-1.879)	0.306
Gender (Male vs. Female)	453	1.164(0.872-1.554)	0.301		
Race (White vs. Asian &Black or African American)	443	0.223(0.103-0.483)	<0.001	0.474(0.063-3.554)	0.468
Tumor tissue site (Extremities vs. Trunk)	354	1.063(0.782-1.443)	0.698		
BRAF status (Mutant vs. Wild Type)	450	0.774(0.589-1.017)	0.066	0.785(0.524-1.176)	0.241
STK17B (High vs. Low)	453	0.694(0.530-0.910)	0.008	0.636(0.430-0.941)	0.024

Table 2. Univariate and multivariate Cox regression analysis of patients' overall survival prediction based on STK17B expression.

melanoma Clark level, Breslow depth, tumor site, BRAF status of SKCM patients (Fig 2). Low STK17B was similarly correlated with worse DSS in T and M Stage, pathologic stage, melanoma Clark level, Breslow depth, melanoma ulceration, tumor site, BRAF status, age, race, gender, radiation therapy of SKCM patients (Fig 3). In addition, the prognostic value of STK17B in SKCM subgroups was visualized (Fig 4). There were significant differences in subgroups of TNM stage, melanoma ulceration, pathologic stage, radiation therapy, gender, age, Breslow depth, BRAF status, indicating that STK17B expression level could impact the prognosis in SKCM patient with different pathological stages.

Table 3.	Univariate and multivariate	Cox analysis of patients'	disease-specific survival	prediction based on §	STK17B expression
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Characteristics	Total (N)	HR (95% CI) Univariate analysis	P value Univariate analysis	HR (95% CI) Multivariate analysis	P value Multivariate analysis
T stage (T3&T4 vs. T1&T2)	353	1.842(1.308-2.594)	< 0.001	0.904(0.508-1.608)	0.731
N stage (N1&N2&N3 vs. N0)	393	1.620(1.179-2.227)	0.003	4.743(1.079-20.844)	0.039
M stage (M1 vs. M0)	421	2.013(1.059-3.828)	0.033	1.943(0.654-5.770)	0.232
Pathologic stage (Stage III &Stage IV vs. Stage I &Stage II)	402	1.495(1.093-2.045)	0.012	0.439(0.097-1.980)	0.284
Melanoma Clark level (IV&V vs. I&II&III)	307	2.075(1.419-3.035)	< 0.001	1.329(0.791-2.233)	0.283
Breslow depth (>3 vs. $< = 3$)	347	2.213(1.580-3.099)	< 0.001	1.718(1.001-2.949)	0.050
Melanoma ulceration (Yes vs. No)	306	1.949(1.369-2.775)	< 0.001	1.435(0.931-2.213)	0.102
Radiation therapy (Yes vs. No)	441	0.966(0.667-1.400)	0.856		
Age (>60 vs. < = 60)	447	1.728(1.278-2.337)	< 0.001	1.205(0.781-1.859)	0.399
Gender (Male vs. Female)	447	1.151(0.847-1.564)	0.368		
Race (White vs. Asian &Black or African American)	437	0.456(0.144-1.450)	0.183		
Tumor tissue site (Extremities vs. Trunk)	350	1.086(0.783-1.505)	0.622		
BRAF status (Mutant vs. Wild Type)	444	0.785(0.586-1.051)	0.104		
STK17B (High vs. Low)	447	0.678(0.508-0.904)	0.008	0.595(0.396-0.894)	0.013





https://doi.org/10.1371/journal.pone.0263311.g002



Fig 3. The influence of STK17B and other clinicopathologic characteristics on the disease-specific survival in skin cutaneous melanoma patients.

B Trans 21 (27) 0.530(359-0.99) 0.044 No 225 (60) 0.530(359-0.99) 0.044 Mo 225 (60) 0.530(359-0.97) 0.027 Mo 116 (40) 0.530(359-0.97) 0.027 Mo 216 (20) 0.020(359-0.97) 0.027 Mo 116 (40) 0.530(359-0.97) 0.077 Standings (10) 116 (20) 0.570(052-0.98) 0.077 Standings (10) 117 (20) 0.570(052-0.98) 0.077 Standings (10) 117 (20) 0.570(052-0.98) 0.077 Standings (11) 0.570(052-0.98) 0.071 0.072 Standings (11) 127 (20) 0.570(052-0.98) 0.071 No 210 (40) 0.570(052-0.98) 0.071 No 225 (60) 0.880(053-0.17) 0.071 No	А	Characteristics	N (%)	Hazard Ratio (95% CI)		P value
B Characteristics 117 (2) 0.528(0.352-0.918) 0.024 0.024 Mater 222 (00) 0.728(0.572-1.180) 0.047 0.047 Mater 0.047 0.558(0.352-0.918) 0.047 0.033 Mater 0.047 0.058(0.352-0.277) 0.047 0.033 Mater 0.047 0.058(0.352-0.277) 0.047 0.033 Mater 0.058(0.353-128) 0.047 0.057 0.037 Mater 0.048(0.047-027) 0.047 0.037 0.047 Mater 0.017(0.057-107) 0.047 0.037 0.047 Mater 0.018(0.327-0.028) 0.017(0.357-117) 0.047 0.027 Mater 0.018(0.027-0.028) 0.017(0.017-102) 0.017(0.017-102) 0.017 0.017 Mater 0.256(0.0000 0.018(0.027-0.028) 0.017 0.027 0.017 Mater 0.256(0.0000 0.018(0.027-0.028) 0.017 0.027 0.017 0.021 0.017 0.021 0.017 0.021 0.017		T stage				
B C C C C C C C C C C C C C C C C C C C		T1&T2	117 (33)	0.529(0.305-0.918)		0.024
National Accession 122(60) 221(0) 12-11(0) 10 1		T3&T4	241 (67)	0.795(0.550-1.149)		0.222
B Construction N(n) 17(44) 25(1) 25(1) 25(2) 25(2) 10(1) 10(N stage	222 (56)	0.752(0.612-1.105)		0.147
Mathematical Mathematin Mathematin Mathematical Mathematical Mathematical Mathematical		N18N28N3	176 (44)	0.551(0.355-0.855)		0.008
Methodna uceration Methodna uceration 0.03 <th0.03< th=""></th0.03<>		M stage MD	404 (95)	0.644(0.484-0.857)		0.002
Visit (16) (ci) (17)		Melanoma ulceration No	144 (46)	0.585(0.368-0.927)		0.023
Bit State 21 (62) 21 (62) 21 (62) 0 (62) 0 (62) Personal State 75 (7) 0 (62) (62) - 0 (8) 0 (62) 0 (62) Ves 75 (7) 0 (62) (62) - 0 (8) 0 (62) 0 (62) Ves 75 (7) 0 (62) (22) - 0 (8) 0 (62) 0 (7) 0 (7) Ves 75 (7) 0 (7) (0 (22) - 0 (8) 0 (7) <td></td> <td>Yes</td> <td>166 (54)</td> <td>0.812(0.512-1.287)</td> <td></td> <td>0.376</td>		Yes	166 (54)	0.812(0.512-1.287)		0.376
Base intersequence 101 (PT) 2.22(0) 346-0.897) 0 No 372 (R2) 0.87(0) (22-0.819) 0.012 Ves 75 (17) 0.85(0) (22-0.819) 0.97 Ves 75 (17) 0.97 (0.25-0.117) 0.98 Ves 75 (17) 0.78 (0.25-0.119) 0.97 Ves 76 (19) 0.77 (0.27-0.102) 0.97 Ves 76 (19) 0.78 (0.250-119) 0.97 Ves 22 (10) 0.800 (250-119) 0.97 Ves 75 (17) 0.78 (0.250-119) 0.97 Ves 75 (17) 0.78 (0.250-1		Pathologic stage Stage I&Stage II	216 (53)	0.678(0.454-1.012)		0.057
Baladion fremary 372 (87) 0 571(0 522-0 18)		Stage III&Stage IV	191 (47)	0.528(0.346-0.807)		0.003
Constant Consta		Radiation therapy No Yes	372 (83)	0.679(0.502-0.918)		0.012
Lensale 17(2(8) 087(0(25)-187) Heads 21(2) 077(0(25)-187) +-00 210(40) 077(0(25)-187) +-00 210(40) 077(0(25)-187) 01 176(5) 07(0(25)-187) BRAF table WT 225(60) 088(0(25)-187) M. 1822N12 M. 1822N12 M. 1822N12 M. 1822N12 M. 1822N12 M. 183(25) 074(0(25)-185) N. 183(25) 074(0(25)-185) N. 183(25) 074(0(25)-187) N. 184(25) 074(0(25)-187) N. 184(25) 074(0(25)-187) N. 184(25) 074(0(25)-187) N. 184(25) 075(0(25)-187) N. 184(25) 075(0(25)		Gender		0.000(0.000 1.200)		0.201
Ape 200 2010 2016(4) 2017(4) 2016(4) 2016(4) 2016(4) 2016(4) 2016(4) 2016(4) 2016(4) 2017(4		Female Male	172 (38) 281 (62)	0.697(0.435-1.117) 0.714(0.512-0.997)		0.134
-280 210 (46) 0.780(510-1149) 0.182 -23 176 (51) 0.780(6270-1622) 0.131 -33 17449 0.897(0.444-1682) 0.131 WT 225 (50) 0.898(0.553-1175) 0.93 WT 225 (50) 0.898(0.553-1175) 0.5 0.918 WT 225 (50) 0.898(0.553-1175) 0.5 0.918 MA 225 (50) 0.898(0.533-1175) 0.5 0.918 MA 227 (67) 0.78(0.526-1187) 0.5 0.011 Nage 0.920 (620-1169) 0.94(0.526-1187) 0.938 0.938 No 220 (65) 0.658(0.341-081) 0.918 0.938 0.938 Mage 396 (65) 0.658(0.341-081) 0.948 0.938 0.938 Stage IISSage IV 118 (47) 0.558(0.341-081) 0.948 0.948 0.948 No 386 (65) 0.658(0.341-081) 0.948 0.948 0.948 0.948 0.948 0.948 0.948 0.948 0.948 <		Age <=60	243 (54)	0.616(0.426-0.889)		0.010
c-3 area 179 (81) 074(0.470-102) 0.121		>60 Breslow depth	210 (46)	0.764(0.510-1.144)		0.192
BRAF status 173 (49) 0.877(0,448-108) 0.112 MA 225 (50) 0.818(0,241-0.82) 0.011 B Churschristics N (%) Hazard Raio (95% C1) P value B Churschristics N (%) Hazard Raio (95% C1) P value Tata 2 227 (67) 0.78(0,232-0.804) 0.021 No 220 (50) 0.58(0,232-0.804) 0.021 No 220 (50) 0.58(0,232-0.804) 0.021 No 220 (50) 0.58(0,232-0.804) 0.023 Mainona ulcantion 174 (47) 0.58(0,232-0.804) 0.023 Mo 398 (55) 0.65(0,421-0.801) 0.023 Stage II83530 0.78(0,428-1.306) 0.023 0.023 Patologic stage 3893 (0.727 0.817) 0.021 0.021 Vie 128 (47) 0.52(0,232-0.842) 0.037 Vie 226 (60) 0.658(0,431-0.807) 0.032 No 226 (60) 0.688(0,437-0.122) 0.041 Vie 128 (47) 0.220 (0.342-1.		<=3	179 (51)	0.716(0.470-1.092)		0.121
WT WT 255 (60) 0.080(0.53-1.176) 0.018 B Characteristics N (%) H.zard Ratio (05% Cl) P value Tatage 115 0.018 0.018 0.018 Tatage 116 (3) 0.516(0.238-0.504) 0.011 0.021 No 220 (60) 0.088(0.529-1.167) 0.021 0.001 Natage 20 (60) 0.028(0.440.564) 0.003 0.003 No 220 (60) 0.028(0.440.564) 0.003 0.003 No 220 (60) 0.028(0.440.564) 0.003 0.038 Matage 162 (67) 0.565(0.237-0.564) 0.003 0.038 Matage 162 (67) 0.565(0.437-0.576) 0.005 0.005 No 162 (7) 0.550(0.437-0.576) 0.005 0.005 No 116 (87) 0.028(0.437-0.576) 0.005 0.005 No 120 (80) 0.570(0.437-0.576) 0.005 0.005 No 120 (80) 0.570(0.237-0.577) 0.033 0		>3 RRAF status	173 (49)	0.697(0.446-1.088)		0.112
Mat 225 (60) 0.018 (0.014-0.022) 0.018 B Characteristics N (%) Hazard Rato (05% Cl) P value Trange Trange Trange 116 (3) 0.516(0.239-0.004) 0.021 N (%) Hazard Rato (05% Cl) P value N (%) Hazard Rato (05% Cl) P value N (%) Hazard Rato (05% Cl) 0.021 N (%) 172 (40) 0.556(0.230-0.054) 0.023 N (%) 162 (30) 0.056(0.31-0.059) 0.056 Packtopic Staps 0.056 (0.32-0.041-0.15) 0.056 N (%) 164 (0.232-1.244) 0.012 Gender 72 (17) 0.046 (0.24-0.102) 0.056 N (%) 172 (60) 0.056 (0.237-0.817) 0.012 Age 275 (62) 0.058 (0.41-0.127) 0.057 -23 177 (64) 0.050 (0.27-0.817) 0.051 N (WT	225 (50)	0.806(0.553-1.175)		0.262
B Characteristics N (%) Hazard Ratio (5% Cl) P value Tratage TATZ 116 (3) 0.514(0.320-0.054) 0.021 0.021 Natage 100 207 (67) 0.784(0.520-1187) 0.230 No 100 207 (67) 0.784(0.520-1187) 0.030 No 120 (66) 0.656(0.341-0.891) 0.033 Maroons utceration 103 (67) 0.556(0.330-0.840) 0.049 No 120 (67) 0.566(0.330-0.840) 0.049 Stage Bitasyse II 214 (63) 0.560(0.37-0.891) 0.017 No 206 (69) 0.790(0.42-1.360) 0.049 0.038 Nate 225 (82) 0.668(0.43-0.1632) 0.011 0.017 No 306 (69) 0.790(0.42-1.440) 0.010 0.017 Nate 225 (82) 0.668(0.43-0.102) 0.019 0.017 Nate 222 (80) 0.790(0.52+1.240) 0.019 0.011 Mate 225 (82) 0.868 (0.40-0.122) 0.049 0.033 Pab <td></td> <td>Mut</td> <td>225 (50)</td> <td>0.618(0.414-0.922)</td> <td></td> <td>0.018</td>		Mut	225 (50)	0.618(0.414-0.922)		0.018
B Characteristics N (%) Hazard Ratio (95% Cl) P value Trage TiaT2 116 (23) 0.516(0.289-0.004) 0.211 TatA1 227 (07) 0.78(0.239-1.004) 0.211 N mape 0.721 (0.231-0.004) 0.230 No 220 (06) 0.086(0.244-1.044) 0.230 N tatpe 0.038 0.055(0.283-0.933) 0.038 Mitanzeau identition No 123 (47) 0.556(0.283-0.933) 0.038 Yes 138 (58) 0.058(0.233-0.833) 0.049 0.038 Bage IllisStage IV 188 (83) 0.058(0.431-0.805) 0.012 No 386 (83) 0.658(0.431-0.805) 0.012 No 386 (83) 0.658(0.237-0.831) 0.037 Age	_				0.5 1 1.5	
C Trange Tra	В	Characteristics	N (%)	Hazard Ratio (95% CI)		P value
TiaT2 116 (3) 0.55(0.239-0.90) 0.20 N range 220 (6) 0.086(0.24-1.187) 0.230 N range 172 (4) 0.55(0.239-0.80) 0.013 M range 172 (4) 0.55(0.239-0.80) 0.013 M range 0.023(0.44-0.85) 0.003 Mo 386 (6) 0.55(0.239-0.83) 0.038 Yes 158 (3) 0.76(0.42-0.85) 0.049 Stage HildStage IV 118 (47) 0.55(0.239-0.83) 0.013 Yes 128 (3) 0.76(0.43-0.95) 0.012 No 386 (3) 0.65(0.43-0.86) 0.012 Yes 128 (41) 0.55(0.237-0.831) 0.012 Yes 177 (5) 0.660(0.37-0.831) 0.049 23 177 (5) 0.660(0.37-0.831) 0.049 241 (64) 0.560(0.27-0.831) 0.049 23 170 (49) 0.680(0.43-1.022) 0.069 241 (64) 0.560(0.27-0.831) 0.049 230 170 (49) 0.680(0.43-1.022) 0.069 241 (64) 0.574(0.278-0.877) 0.159 M		T stage				
Lat. 1* 23 (6) 0. 59(2) <th0. 59(2)<="" th=""> <th0. 59(2)<="" th=""> <th0< td=""><td></td><td>T18T2</td><td>116 (33)</td><td>0.515(0.293-0.904)</td><td></td><td>0.021</td></th0<></th0.></th0.>		T18T2	116 (33)	0.515(0.293-0.904)		0.021
NO 220 (56) 0.58(0.041-0.81) 0.013 M stage 0.023(0.44-0.81) 0.033 M stage 0.032(0.44-0.81) 0.033 Molarona ulcension 0.055(0.383-0.83) 0.033 No 142 (47) 0.556(0.432-0.84) 0.038 Stage IIS stage II 214 (53) 0.656(0.432-0.86) 0.048 Stage IIS stage III 214 (53) 0.656(0.431-0.86) 0.048 Radation theraxy 0.043(0.431-0.816) 0.012 0.044 No 386 (63) 0.658(0.431-0.86) 0.012 0.012 Na 72 (17) 0.658(0.431-0.122) 0.014 0.012 Na 221 (64) 0.658(0.431-0.122) 0.014 0.012 Yes 211 (7 (1)) 0.658(0.431-0.122) 0.014 0.037 Age 212 (62) 0.698(0.431-0.122) 0.014 0.037 Yes 223 (60) 0.574(0.276-0.877) 0.010 0.011 Yes 10.658(0.431-0.122) 0.044 0.011 0.0110 Yes		N stage	237 (67)	0.784(0.526=1.167)		0.230
M : Tage N : Top Description Description Description Md arona uteration 142 (47) 0.55(0.38-0.954) 0.033 Net 152 (53) 0.794(0.422-1.366) 0.038 Pachologic stage 53age 1653bg 11 214 (53) 0.658(0.431-0.966) 0.049 Stage 1653bg 11 214 (53) 0.658(0.431-0.866) 0.049 0.049 Radiation therapy 0.058(0.431-0.867) 0.047 0.012 Ns 72 (71) 0.658(0.431-0.867) 0.047 Rediation therapy 0.058(0.431-0.87) 0.017 0.017 Ne 72 (72) 0.658(0.431-0.82) 0.017 0.017 Age		NO N18N 28N 3	220 (56)	0.696(0.464-1.044)		0.080
MU JB (E) U.24(0.59-0.594) U.24(0.59-0.594) NO 143 (47) 0.555(0.283-0.643) 0.023 Pathologis stage 0.038 0.023 0.049 Stage Hild Stage IV 106 (47) 0.555(0.283-0.643) 0.049 Stage Hild Stage IV 106 (47) 0.552(0.283-0.615) 0.049 Roaddon Therapy 0.049 0.049 0.049 No 208 (63) 0.652(0.237-0.623) 0.049 Pathologis stage 172 (60) 0.760(0.277-0.831) 0.049 Conder 721 (7) 0.656(0.277-0.831) 0.044 -60 206 (69) 0.870(0.524-1.244) 0.044 -760 206 (69) 0.870(0.524-1.244) 0.038 BFAF status 170 (69) 0.650(0.419-1.120) 0.044 Virt 221 (60) 0.870(0.524-1.244) 0.338 BFAF status 170 (69) 0.650 (0.419-1.120) 0.044 No 223 (60) 0.574(0.547-0.587) 0.010 No 223 (60) 0.574(0.547-0.1037) 0.0		M stage	000 (05)	0.000(0.001-0.001)		0.013
No 143 (47) 0.556(0.383-0.843) 0.028 Yes 155 (35) 0.74(0.425-1.365) 0.386 Pathologic stage 214 (43) 0.556(0.431-0.86) 0.049 Stage III.85keg IV 118 (47) 0.552(0.431-0.86) 0.049 Pathologic stage 128 (47) 0.522(0.330-0.55) 0.012 No 386 (83) 0.683(0.40-0.515) 0.012 Yes 72 (17) 0.058(0.437-0.831) 0.049 Age		Mu Melanoma ulceration	388 (82)	0.029(0.404-0.804)		0.003
Pathologic stage 100 (co) 0.148(0.51 - 1.500) 0.049 Steps III.65tep (V) 184 (67) 0.522(0.330 - 0.925) 0.049 Steps III.65tep (V) 188 (67) 0.522(0.330 - 0.925) 0.049 No 386 (83) 0.652(0.41 - 0.915) 0.014 Yes 72 (17) 0.84(0.332 - 1244) 0.114 Male 275 (82) 0.84(0.478 - 0.878) 0.027 App - 0.026(0.431 - 1.165) 0.014 -e00 206 (e0) 0.870(0.241 - 1.46) 0.0301 -e30 241 (64) 0.656(0.478 - 0.873) 0.044 -e30 177 (69) 0.658(0.478 - 0.877) 0.014 -e31 177 (69) 0.658(0.478 - 0.877) 0.345 Mit 223 (60) 0.574(0.276 - 0.877) 0.345 Mit 223 (60) 0.574(0.276 - 0.877) 0.345 Nit At 223 (00) 0.574(0.276 - 0.877) 0.345 Nit App N(%) Hazed Rato (95% CI) P value Teage 115 1.5 0.754(0.567 - 1.077) 0.5 0.754(0.567 - 1.077) 0.5 0.155 0.345		No	143 (47)	0.585(0.383-0.943)		0.028
Stage 16 Stage 11 214 (53) 0.656(0.431-0.866) 0.005 Facadation therapy 0.005 (0.001 0.005 0.005 No 386 (03) 0.658(0.481-0.815) 0.012 No 386 (03) 0.658(0.481-0.815) 0.012 Yes 72 (17) 0.614(0.303-1.244) 0.017 Gender 72 (17) 0.726(0.431-1.165) 0.012 Yes 215 (22) 0.085(0.431-0.132) 0.044 Yes 214 (24) 0.550(0.271-0.831) 0.044 Yes 216 (26) 0.650(0.437-1.022) 0.044 Yes 216 (26) 0.650(0.412-1.244) 0.322 BRAF status 177 (46) 0.650(0.412-1.234) 0.010 VFT 221 (00) 0.674(0.370-0.877) 0.010 VTT 221 (00) 0.674(0.570-0.877) 0.010 VTT 222 (60) 0.674(0.670-0.877) 0.010 VTT 222 (60) 0.832(0.002-1.153) 0.010 No 222 (60) 0.832(0.002-1.153) 0.021 <		Pathologic stage	100 (00)	0.10 (0.102 1.000)		0.000
C Characteristics N (b) Hazard Ratio (95% Cl) P value Tatage 177 (61) 0.678(0.647-0.65% Cl) 0.678(0.647-0.67% Cl) 0.012 Yis 256 (62) 0.689(0.470-0.976) 0.021 0.012 Wis 275 (62) 0.689(0.470-0.976) 0.021 0.021 Age 275 (62) 0.689(0.470-0.976) 0.037 0.031 -600 206 (66) 0.697(0.524-1.244) 0.044 0.032 -763 177 (61) 0.688(0.419-1.122) 0.049 0.335 BFA/F status 170 (66) 0.692(0.547-0.531) 0.010 0.010 UT 271 (60) 0.623(0.549-1.230) 0.010 0.010 UT 271 (60) 0.623(0.549-1.230) 0.010 0.010 UT 171 (23) 0.676(0.443-1.037) 0.015 0.155 No 223 (65) 0.633(0.002-1.153) 0.015 0.015 N HAL22013 177 (44) 0.754(0.589-0.586) 0.021 0.054 No 223 (65) 0.754(0		Stage I&Stage II Stage III&Stage IV	214 (53)	0.656(0.431-0.999)		0.049
No. 386 (83) 0.653(0.481-0.915) 0.012 Visis 73 (17) 0.514(0.303-1.244) 0.176 Gender 72 (26) 0.766(0.431-1.155) 0.176 Age		Radiation therapy	100 (41)	0.022(0.000 0.020)		0.000
Cender Female 172 (36) 0.706(0.431-1185) 0.134 Male 275 (82) 0.88(0.378-0.873) 0.007 Age		No Yes	368 (83) 73 (17)	0.663(0.481-0.915) 0.614(0.303-1.244)		0.012
Maie 275 (82) 0.884(0.478-0.276) 0.037 Age <		Gender Female	172 (38)	0.708(0.431-1.165)		0.174
¹² -650 ¹² -650 ¹² -650 ¹² -650 ¹² -650 ¹² -65 ¹² -75 ¹²		Male	275 (62)	0.684(0.478-0.978)		0.037
>80 206 (66) 0.870(6,524+1.244) 0.332 Previous degin		<=60	241 (54)	0.560(0.377-0.831)	→	0.004
c-3 177 (61) 0.088(0.431-0.122) 0.088 BRAF status 170 (66) 0.082(0.540-1.23) 0.138 BRAF status 170 (66) 0.082(0.540-1.23) 0.138 WT 221 (60) 0.022(0.540-1.23) 0.010 Mat 223 (50) 0.574(0.376-0.877) 0.010 1.5 1.5 0.010 0.023(0.540-1.23) 0.010 Characteristics N (%) Hazard Ratio (95% Cl) P value Tatage 11.5 0.078(0.641-1.037) 0.015 Tatage 11.12 0.078(0.611-1.08) 0.015 N mage N0 223 (56) 0.833(0.002-1.15) 0.015 N mage N0 223 (56) 0.833(0.002-1.15) 0.015 N mage N0 223 (56) 0.754(0.589-0.958) 0.021 Met arona alcension N0 144 (46) 0.738(0.507-1.077) 0.116 Stage HS3bage II 216 (53) 0.764(0.549-1.089) 0.116 0.498 Stage HS3bage II 216 (53) 0.632(0.697-1.222)		>60 Breslow depth	206 (46)	0.807(0.524-1.244)		0.332
P3 P10(e9) Useque 3(e1,12g) Useque 3(e1,12g) <thuseque< td=""><td></td><td><=3</td><td>177 (51)</td><td>0.668(0.433-1.032)</td><td></td><td>0.069</td></thuseque<>		<=3	177 (51)	0.668(0.433-1.032)		0.069
WT 221 (60) 0.823(0.54-1.23r) 0.945 Mat 223 (50) 0.574(0.376-0.877) 0.910 C Characteristics N (%) Hazard Ratio (85% Cl) P value Tatage 1.5 0.073 0.073 0.073 Tatage 0.0730(0.571-1063) 0.0730(0.449-1037) 0.073 0.073 Natage 0.0790(0.571-1063) 0.0730(0.489-1065) 0.021 0.155 Natage 10.7764(0.489-1065) 0.021 0.021 0.033 Natage 0.0790(0.489-1065) 0.021 0.021 0.033 Matage 10.7764(0.449-1065) 0.021 0.021 0.021 Matage 0.0790(0.489-0.698) 0.021 0.021 0.033 Matage 10.777(44) 0.720(0.484-0.698) 0.048 0.048 Badge IdStage II 216 (53) 0.764(0.546-1.069) 0.187 0.048 Stage IdStage II 216 (53) 0.521(0.637-1.056) 0.197 0.887 Radiation therapy No 0.222(0.038-1.057) 0		>3 BRAF status	170 (49)	0.688(0.419-1.128)		0.138
Mile 223 (b0) 0.314(0.519-0.517) 0.314(0.519-0.517) C Characternitics N (%) Hazard Ratio (95% C1) P value Tetage T1612 117 (23) 0.676(0.449-1.037) 0.073 Tata12 117 (23) 0.676(0.449-1.037) 0.073 0.165 N (%) Hazard Ratio (95% C1) P value 0.021 Natage 10.220 (66) 0.833(0.802-1.153) 0.021 NO 223 (66) 0.833(0.802-1.153) 0.021 Mitanzoa ulcenzion 0.056 0.754(0.589-0.958) 0.021 Mitanzoa ulcenzion 0.656 0.754(0.589-0.958) 0.021 Mitanzoa ulcenzion 0.656 0.754(0.589-0.958) 0.021 Mazaon ulcenzion 106 (64) 0.230(0.546-1.222) 0.338 Stage Hit3Stage IV 116 (64) 0.820(0.546-1.923) 0.116 Stage Hit3Stage IV 116 (64) 0.820(0.546-1.923) 0.388 Moin chrony 73 (83) 0.821(0.837-1.056) 0.117 No 372 (83) 0.821(0.837-1.056) <		WT	221 (50)	0.823(0.549-1.234)		0.345
0.5 1 1.5 C Characteristics N (%) Hazard Ratio (05% C1) P value Tatage 1117 (23) 0.678(0.443-1.037) 0.073 0.073 Tatage 11312 117 (23) 0.78(0.571-1.083) 0.073 0.155 N range 1078(0.571-1.083) 0.073 0.073 0.155 0.155 N range 1072(0.527-1.083) 0.073 0.073 0.073 0.073 No 223 (60) 0.833(0.002-1.153) 0.021 0.054 0.024 Mol 223 (60) 0.754(0.589-0.956) 0.021 0.054 0.024 Mol scons ulcension 405 (65) 0.754(0.589-0.956) 0.021 0.054 0.021 No 108 (54) 0.202(0.545-1.026) 0.049 0.338 0.049 Radiation therapy No 0.021(0.657-1.056) 0.049 0.166 Radiation therapy No 0.021(0.657-1.056) 0.049 0.129 Nike 222 (22) 0.0380(0.055-1.056) 0.129			223 (50)	0.514(0.576-0.677)		0.010
Characteristics N(%) Histola (480, (454, L)) Product Tagge Tagge Tagge Tagge Tagge 11872 117 (23) 0.0730(0.442-1.037) Natage NO NO 222 (66) 0.0830(0.00-1.153) Natage NO No 222 (66) 0.0830(0.00-1.153) Natage NO No 222 (66) 0.0790(0.489-1.006) Natage NO No 222 (66) 0.0790(0.489-1.006) Natage NO No 2330(0.00-1.153) Natage No No No No No No No No No No	C				0.5 1 1.5	
T stape 0.754(0.443-1.037) 0.073 T sta 1 242 (87) 0.759(0.571-1.033) 0.155 N rate 0.073 0.156 N rate 0.073 0.074 NO 223 (86) 0.833(0.627-1.033) 0.013 N Rate 0.074 0.010 (489-1.066) 0.021 M stape 0.074 0.054 0.021 M0 405 (65) 0.754(0.582-0.656) 0.021 M6 0.023 (0.507-1.077) 0.118 0.339 Pachologic stage 0.520 (0.547-1.232) 0.339 Stape 165 tage III 216 (63) 0.764(0.548-1.232) 0.549 Rodation therapy 0.049 0.549 0.549 Rodation therapy 0.521(0.687-1.056) 0.127 0.677 Rodation therapy 0.521(0.687-1.056) 0.127 0.579 Rodation therapy 0.520 (0.561-1.032) 0.506 0.127 Rodation therapy 0.520(0.561-1.032) 0.520 0.520 Rodation therapy 0.560 0.127 0.520 0.520 Rotaction therapy 0.560 0.520	C	Characteristics	N (%)	Hazard Ratio (95% CI)		P value
111123 00110(0.052-11.037) 00110(0.052-11.037) 111123 00110(0.052-11.037) 00110(0.052-11.037) No 223 (60) 0830(0.002-11.153) 0.271 NO 1177 (44) 0.710(0.489-1.068) 0.251 Matage 0050 0.754(0.589-0.956) 0.221 Matage 0050 0.754(0.589-0.956) 0.221 Matage 0.020(0.545-1.232) 0.338 0.238 Matage 116 (54) 0.220(0.545-1.232) 0.338 Stage 16530ge II 216 (53) 0.740(0.545-1.089) 0.498 Radiation therapy 0.221(0.637-1.056) 0.116 0.989 Radiation therapy 0.221(0.637-1.056) 0.127 0.989 No 373 (83) 0.21(0.637-1.056) 0.127 0.989 Radiation therapy 0.22(0.038-1.077) 0.220 0.388 0.129 0.129 No 373 (83) 0.21(0.637-1.056) 0.129 0.129 0.129 0.129 0.129 0.129 0.129 0.129 0.129 0.129		T stage	117 (99)	0.679/0.442-4.0271		0.070
N stage N stage N stage N 1812/2813 N 18		T3&T4	242 (67)	0.790(0.571-1.093)		0.155
NU 224 (56) 0.03840 (62-1133) 0.221 (133) 0.221 (134) 0.054 (134)		N stage	000 (50)	0.000/0.000 4.450		0.074
M stage M0 Md anona ulceration No Nes Stage 16 Stage 11 Stage 16 Stage 11 Parbologic stage Stage 16 Stage 11 Parbologic stage No Stage 16 Stage 11 Parbologic stage Parbologic stage No Stage 16 Stage 11 Parbologic stage Parbologic stage No Parbologic stage No Parbologic stage No Parbologic stage Parbologic stage No Parbologic stage No Parbologic stage No Parbologic stage No Parbologic stage No Parbologic stage No Parbologic stage No Parbologic stage Parbologic stage No Parbologic stage Parbologic stage 10 Parbologic stage Parbologic stage 10 Parbologic stage Parbologic stage Parbologic stage 10 Parbologic stage Parbologic stage Parbol		N18N28N3	177 (44)	0.701(0.489-1.006)		0.054
Mel soons utcration 144 (46) 0.738(0.507-1.077) 0.16 No 0.738(0.507-1.077) 0.16 0.338 Parbologic stage 510g (45,07-0.077) 0.338 0.338 Stage 1653toge III 216 (53) 0.764(0.546-1.089) 0.116 Radiation therapy 0.021(0.637-1.056) 0.127 0.488 Radiation therapy 0.22(0.048-0.089) 0.197 0.097 No 373 (83) 0.22(0.048-1.089) 0.197 0.097 No 373 (83) 0.22(0.081-1.071) 0.097 0.097 No 75 (17) 0.52(0.081-1.071) 0.388 0.127 Wes 75 (17) 0.52(0.081-1.071) 0.388 0.189 Age		M stage MD	405 (95)	0.754(0.593-0.958)		0.021
No 144 (46) 0.738(0.507-1077) 0.118 Yes 780/0.605-1232) 0.339 Parbologic stage 126 (53) 0.764(0.546-1.032) 0.939 Stage III.85age IV 112 (47) 0.702(0.484-1.232) 0.919 Radiation threapy 0.018 0.821(0.637-1.056) 0.179 No 373 (83) 0.821(0.637-1.056) 0.170 Vies 75 (17) 0.822(0.881-1071) 0.987 Gender 7640 0.948(0.579-1.236) 0.987 Age 0.118 0.129 0.2800 Age 0.120 (64) 0.830(0.050-1.060) 0.129 Age 0.126 (64) 0.786(0.538-1.037) 0.088 Haie 2.92 (52) 0.830(0.057-1.060) 0.129 Age 0.128 0.244 (4) 0.786(0.533-1.057) 0.008 -000 210 (64) 0.758(0.533-1.057) 0.100 0.280 -260 2.44 (44) 0.758(0.532-1.057) 0.100 0.158 -261 176 (51) 0.758(0.532-1.057)		Melanoma ulceration	400 (00)	0.104(0.000 0.000)		0.021
Partologic stage Color		No	144 (46)	0.739(0.507-1.077) 0.820(0.545-1.232)		0.116
Stage Id Stage II 216 (53) 0.764(0.546-1.069) 0.116 Stage Id Stage II 216 (73) 0.074(0.546-1.069) 0.649 Radation theracy 0.049 0.549 0.549 No 373 (83) 0.821(0.637-1.056) 0.127 Ves 75 (17) 0.822(0.637-1.056) 0.949 Age 0.049 0.368 0.368 Age 0.22(0.210-1.056) 0.368 0.127 Age 0.2600 (0.057-1.068) 0.368 0.368 >800 244 (64) 0.769(0.569-1.038) 0.068 >800 210 (46) 0.813(0.567-1.167) 0.260 -3 179 (61) 0.740(0.489-11.24) 0.159 BRAF status 0.73(69) 0.74(0.489-11.24) 0.159 Wr 226 (50) 0.740(0.522-1.053) 0.569 Mut 226 (50) 0.750(0.522-1.053) 0.569		Pathologic stage	100 (04)	0.010(0.040 1.101)		0.000
Radiation therapy Cli (1/) Cli (2/) Cli (2/) <td>Stage I&Stage II Stage III&Stage IV</td> <td>216 (53)</td> <td>0.764(0.546-1.069)</td> <td></td> <td>0.116</td>		Stage I&Stage II Stage III&Stage IV	216 (53)	0.764(0.546-1.069)		0.116
No 373 (83) 0.621 (0.877-10.56) 0.127 Vies 75 (71) 0.921 (0.877-10.56) 0.087 Gender 0.946(0.579-12.86) 0.987 Female 172 (88) 0.948(0.579-12.86) 0.398 Male 262 (22) 0.9303 (0.65-10.86) 0.129 Age . 0.260 0.260 -80 244 (64) 0.786 (0.569-10.86) 0.080 -80 170 (64) 0.756 (0.533-10.57) 0.060 erstow degm . 0.158 158/4 VT 225 (50) 0.914 (0.82-1.783) 0.588 Md. 226 (50) 0.783 (0.52-1.056) 0.588		Radiation therapy	102 (11)	0.102(0.101 0.000)		0.010
Gender 12 (2) 0.4 (46) (2370-126) 0.6 (46) (2370-126) 0.6 (46) (2370-126) Finansie 172 (26) 0.6 (46) (2370-126) 0.2 66 0.129 Age 262 (62) 0.6 (300-108) 0.0 66 0.2 66 Financia 179 (46) 0.8 (0.6 57-1.1 57) 0.2 66 Folo 210 (46) 0.8 (0.6 57-1.1 57) 0.2 66 Folo 210 (46) 0.8 (0.6 57-1.1 57) 0.2 50 Folo 210 (46) 0.8 (0.6 57-1.1 57) 0.2 50 Folo 179 (46) 0.7 4 (0.4 88-1.1 24) 0.1 50 FOLO 74 (0.4 88-1.1 24) 0.1 50 0.5 66 WT 22 5 (50) 0.7 4 (0.4 80-1.2 83) 0.5 66 Mut. 22 6 (50) 0.7 8 (0.5 22-1.0 56) 0.1 60		No Yes	373 (83) 75 (17)	0.821(0.637-1.058) 0.622(0.381-1.071)		0.127
remae 172 (80) 0.944(0.57-128) 0.386 Male 22 (22) 0.0300 (055-10.030) 0.129 Age		Gender				
Age 0.789(0,589-10.38) 0.789(0,589-10.38) 0.086 >80 210 (46) 0.813(0,571-1157) 0.086 c=3 179 (51) 0.750(0,533-1057) 0.100 >3 172 (49) 0.741(0,489-11.24) 0.188 BRAF status WT 225 (50) 0.914(0,682-1.283) 0.598 Mut 226 (50) 0.783(0,552-1.056) 0.102		Female Male	172 (38) 282 (62)	0.846(0.579-1.236) 0.803(0.605-1.066)		0.386
<		Age	(04)			
Brestow deg/m		<=60 >60	244 (54) 210 (46)	0.769(0.569-1.038) 0.813(0.571-1.157)		0.086
Image: Normal Status Image: No		Breslow depth	170 /543	0.750/0.622-4.0571		0.100
ERAF status WT 225 (50) 0 0 14(0.882-1.283) Mut 226 (50) 0.783(0.552-1.056)		>3	178 (51)	0.741(0.488-1.124)		0.100
Mut 228 (50) 0.783(0.552-1.056) - 0.102		BRAF status WT	225 (50)	0.914(0.662-1.263)		0.586
· · · · · · · · · · · · · · · · · · ·		Mut	226 (50)	0.763(0.552-1.056)	→	0.102
		-			_, ,	

Fig 4. Prognostic value of STK17B in skin cutaneous melanoma subgroups. Multivariate analysis in overall survival (**A**), disease-specific survival (**B**), and progression-free interval (**C**) of SKCM project.

3.5. Nomogram construction based on STK17B expression and clinicopathological factors

Based on the results of multivariate analyses with the Cox regression model, we integrated both STK17B expression and other clinicopathological prognostic factors, then constructed nomograms to better predict 1-year, 3-year and 5-year OS, DSS, and PFI of SKCM patients (Fig 5). Higher total points indicated a worse outcome. The C-index for OS, DSS, and PFI prediction were 0.699(0.673–0.725), 0.696(0.668–0.724), and 0.685(0.661–0.708) respectively. The calibration curve for the probability of survival at 1-, 3-, and 5-year showed good agreement between the prediction by nomogram and actual observation. These nomogram-based results demonstrated a good accuracy for predicting the 1-, 3-, or 5-year survival of SKCM patients.

3.6. The correlation between STK17B expression and tumor-infiltrating immune Cells

We analyzed 24 types of infiltrating immune cells by ssGSEA method in SKCM, and the association between STK17B expression and 24 types of infiltrating immune cells by Spearman correlation. The results showed that STK17B expression level was significantly positively correlated with infiltrating levels of CD8 T cells (R = 0.346, P < 0.001), Treg (R = 0.307, P < 0.001), Th2 cells (R = 0.488, P < 0.001), Th1 cells (R = 0.515, P < 0.001), Tcm (R = 0.513, P < 0.001), Thelper cells (R = 0.702, P < 0.001), T cells (R = 0.523, P < 0.001), Cytotoxic cells (CD8+) (R = 0.523, P < 0.001), B cells (R = 0.462, P < 0.001) (Fig 6A). It has been revealed the relationship of STK17B expression and the infiltration levels of 24 immune cells (Fig 6B). Moreover, we analyzed the differences of infiltration levels of immune cells between STK17B high- and low-expression groups (Fig 7A). Immune score analysis showed that STK17B expression was positively correlated with immune score, stromal score, and ESTIMATE score (Fig 7B–7D). Accordingly, these three scores were higher in patients with STK17B high-expression than low-expression (S1A–S1C Fig). STK17B expression was different in different immune subtypes (S1D Fig). These findings suggested that STK17B played an important role in immune cells infiltration of tumor microenvironment in SKCM.

3.7. Identification of DEGs between STK17B high- and low-expression groups

To gain the insight of STK17B biological meaning in SKCM, an RNA-seq analysis was used to compare the gene expression profiles of STK17B high- and low-expression groups in TCGA database. Based on "edgeR" in R software, 547 genes expression associated with STK17B in SKCM were screened out, including 372 up-regulated genes and 175 down-regulated genes (Fig 8A). The top 12 significant genes positively and negatively correlated with STK17B were shown in the heat map (Fig 8B).

3.8. Functional enrichment of STK17B-related genes in SKCM

To further explore the function of the DEGs and identify key candidate pathways, GO functional analysis and KEGG pathway analysis was performed. We selected the top GO terms of the molecular function, biological process, cellular component (Fig 8C–8E). The enriched terms included cytokine receptor binding, glycosaminoglycan binding, cytokine activity, epidermis development, skin development, epidermal cell differentiation, keratinocyte differentiation, cornified envelope, intermediate filament cytoskeleton (S1 Table). In the TCGA cohort, KEGG pathways, such as cytokine-cytokine receptor interaction, estrogen signaling pathway, viral protein interaction with cytokine and cytokine receptor, which were most significantly



Fig 5. Nomograms of prognostic model and calibration curve. Nomogram models of overall survival (**A**), disease-specific survival (**B**), and progression-free interval (**C**) with respect to the BRAF status, melanoma Clark level, tumor tissue site, gender, Breslow depth, T stage, M stage, N stage, age, pathologic stage, radiation therapy, race, melanoma ulceration and STK17B gene expression.



Fig 6. The correlation between infiltration levels and STK17B expression in SKCM patients. (A) STK17B expression was positively correlated with the infiltration levels of CD8 T cells, Treg, Th2 cells, Th1 cells, Tcm, T helper cells, T cells, Cytotoxic cells (CD8+), and B cells; (B) the relationship of STK17B expression and the infiltration levels of 24 types immune cells.



Fig 7. Infiltration level of immune cells and immune score analysis. (A) Comparison of different immune cells in STK17B high- and lowexpression groups in the SKCM cohorts from The Cancer Genome Atlas database; the correlation between STK17B expression and stromal score (B), immune score (C) and ESTIMATE score (D).



Fig 8. Differential expression genes between STK17B high- and low-expression groups. (A) A volcano plot of differential expression genes between STK17B high- and low-expression groups; **(B)** a heat map of the top 12 significant genes positively and negatively correlated with STK17B; Gene Ontology **(C-E)** and Kyoto Encyclopedia of Genes and Genomes **(F)** analyses of STK17B related differential expression genes.

enriched in SKCM patients with higher expression compared to lower expression (Fig 8F, S1 Table). GO and KEGG analysis revealed that STK17B might mediate the process of cellular differentiation and immunity, which consisted with the results of immune infiltration analyses.

3.9. Gene set enrichment analysis (GSEA) and a PPI network

GSEA was performed between STK17B high- and low-expression group. Results of GSEA revealed significant differences in MSigDB collection enrichment (c5.all.v7.0.symbols.gmt [Gene ontology]). As shown in Fig 9, the results suggested that high expression of STK17B may be highly enriched in regulation of innate immune response, leukocyte differentiation, lymphocyte migration, T cell activation, lymphocyte differentiation, regulation of lymphocyte activation, regulation of vasculature development, and positive regulation of cell adhesion, revealing that STK17B was related to immunity and cancer development. To further investigate the role of the STK17B in the development of SKCM, we constructed a PPI network by STRING to evaluate the interaction between the relevant genes (Fig 9).

4. Discussion

SKCM is still a refractory disease, which is also the leading cause of death from skin cancer [20]. The advanced patients' survival has been prolonged by current clinical therapeutic methods. Nevertheless, many patients were observed subsequent resistances to the drugs and developing progressive diseases [21]. The 5-year survival of patients with metastatic melanoma is only 23%, less than a quarter of patients with localized disease [22]. It's urgent to identify more effective diagnostic and prognostic biomarkers. STK17B, which has high expression in lymphoid tissue, has been indicated associated with hepatocellular carcinoma and some other cancers by previous studies. However, the clinical value of STK17B for SKCM is undefined. In this research, we mainly investigated the role of STK17B on prognosis, clinicopathologic features, and immune-related characteristics of SKCM.

STK17B is a death-associated protein kinase and involved in apoptosis in a variety of cell types [23, 24]. In our study, we analyzed STK17B expression data in SKCM samples obtained from TCGA database and found that the low expression level of STK17B was relevant to a poor prognosis. Radiation therapy is widely used as an adjuvant therapy for patients with advanced melanoma. It was found that radiation could increase tumor associated antibodies and the diversity of T-cell receptor [25, 26]. Our research revealed that STK17B expression was closely associated to the infiltration level of immune cells and regulated immune-related progresses. STK17B could not be a potential biomarker to predict prognosis in SKCM patients with radiation therapy because STK17B-related immune progress was changed under radiation treatment. Previous studies have demonstrated that STK17B could be an independent prognostic factor in chronic lymphocytic leukemia. The results also showed that the low STK17B expression level was significantly linked to the shorter patients' OS [27]. On the contrast, STK17B is highly expressed in hepatocellular carcinoma (HCC) tissues, which was considered predicting a poor prognosis [6]. Different effects of STK17B expression level on prognosis might be due to the disparate pathogenesis and the correlation between STK17B and prognosis in diverse diseases need more statistical verification. Analyses of this research revealed that expression of STK17B was in obvious connection with OS and DSS for SKCM so that it could be regarded as a new prognostic biomarker of this cancer for further clinical study.

As we know, clinicopathological features presented by patients are closely related to the development and prognosis of the disease. To further explore the value of STK17B in the diagnosis and treatment of SKCM, we analyzed the relationship of STK17B with



Fig 9. Gene set enrichment analysis of significantly enriched gene sets between STK17B high- and low-expression groups and a protein-protein interaction network. (A) Gene set enrichment analysis of significantly enriched gene sets between STK17B high- and low-expression groups; (B) a protein-protein interaction network of STK17B related differential expression genes.

clinicopathological features. The results indicated that STK17B was correlated with T stage, Breslow depth and radiation therapy in SKCM patients. It has been identified that high expression level of STK17B in HCC was strikingly related to poor clinicopathological feature, including tumor size, TNM stage, and venous invasion [6]. Similarly, the significant correlations can also be found between the abnormal expression of STK17B and clinicopathologic variables in non-Hodgkin's lymphoma [28]. Above results suggested that STK17B was an important factor influencing clinicopathological characteristics and could be a possible tumor biomarker and drug target. To a certain extent, the nomogram could predict the prognosis of SKCM patients.

Tumor-infiltrating immune cells constitute a part of the tumor microenvironment and play an important role in regulating tumor development and progression. Previous researches have revealed that STK17B highly expresses in B and T cells and negatively regulates activated T cells [29, 30]. In this research, we noticed that STK17B expression level was positively correlated with infiltration levels of Th cells, T cells, Th1 cells, Tcm, and Th2 cells by estimating the association of STK17B expression and immune cells. Previous study revealed that IL-9-producing CD4+ T cells, a type of T helper cells, had potent abilities in eradicating melanoma [31]. Results of our study showed that STK17B expression was significantly associated to infiltration level of T helper cells. According to prognosis analysis results of our research, higher STK17B expression indicated longer overall survival and disease-specific survival. The underlying mechanism might be high STK17B expression leading to high infiltration level of immune cells which contribute to resist tumor cells. Therefore, high STK17B expression suggest good prognosis. It has been found that STK17B affected autoimmune diseases via regulating T cell survival [32]. Besides, the breast cancer cell line with depletion of STK17B retarded tumorigenesis and inhibited tumor growth in a xenograft model [5]. However, Benjamin A. Edwards, et al. deemed that STK17B was not a necessary tumor-inhibiting factor or an oncogene. Take into consideration that STK17B expresses differently in humans and mice, the mechanism and consequences should also be distinguishing. It is also possible that tissue specificity causes STK17B to have different mechanisms in SKCM. STK17B mediates SKCM related immune mechanisms in other undiscovered ways. After all, it's undoubted that STK17B is significantly linked to immunological process.

Staphylococcus aureus (S. aureus) is a common pathogenic factor of bloodstream infection, which has a strong impact on mortality of cancer patients [33]. However, it has also been clarified that S. aureus infection is associated with low melanoma risk. The mechanism could be that immune response caused by S. aureus infection also identifies tumor cells and kill them during defensing S. aureus, suggesting that S. aureus infection is closely linked to morbidity and mortality in melanoma patients [34]. The enrichment results of our study revealed that differential expression of STK17B in melanoma patients was related to S. aureus infection. Our study also demonstrated the correlation between STK17B expression and immune-related progress in melanoma patients. Different expression level of STK17B might regulate the sensibility to S. aureus and influence prognosis of SKCM patients.

All above analyses of this research were based on the information from TCGA and GTEx databases, which emphasized on theoretical research. It is not known whether our results consist with experimental verification. In vitro and in vivo experiments are required to clarify the influence of STK17B on the development of SKCM. The underlying mechanisms need to be ascertained to prove the value of STK17B in settling clinical practical problems and rationalize the different results of other studies. On this basis, the therapeutic effect of STK17B on SKCM can be further studied.

In summary, we studied the relationship between STK17B and SKCM for the first time. We discovered that lower STK17B expression was connected with shorter OS and DSS in SKCM patients. Moreover, this gene was likewise related to clinicopathologic features and immune-related characteristics of SKCM. These results connote STK17B may be a molecular targets and lead to new discovery of treatments for SKCM.

Supporting information

S1 Fig. Immune score of STK17B expression and immune subtype analysis. Stromal score (**A**), immune score (**B**), and ESTIMATE score (**C**) of STK17B high- and low-expression groups; (**D**) STK17B expression level in different immune subtypes. (TIF)

S1 Table. Enrichment analyses of STK17B-related genes in skin cutaneous melanoma. (DOCX)

Acknowledgments

The authors sincerely acknowledge the Helixlife for some bioinformatics approaches to explore the databases.

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