Low expression of SEMA4D as a potential predictive molecular marker of poor survival in patients with melanoma combined with liver cancer

XIANG LIU[1], CHONG ZHANG[1], WU-HAN YANG[1], SHENG-CHAO LI[1], RUI-FENG WANG[2], YI-BIN ZHANG[2] and ZHI-LEI ZHANG[1]

[1]Department of Hepatobiliary Surgery, The Fourth Affiliated Hospital of Hebei Medical University, Shijiazhuang, Hebei 050011; [2]School of Basic Medicine, Hebei Medical University, Shijiazhuang, Hebei 050017, P.R. China

Received July 8, 2022; Accepted November 21, 2022

DOI: 10.3892/ol.2023.13746

Abstract. This study explored the correlation between semaphorin 4D (SEMA4D) and the prognosis and survival time of patients with melanoma combined with liver cancer. A total of 272 patients were recruited, and clinical and follow-up data were recorded. The expression levels of SEMA4D and SEMA3B were determined. Pearson's χ^2 test and Spearman's rank correlation coefficient were used to analyze the relationship between prognosis and the assessed parameters of melanoma patients. Univariate and multivariate Logistic regression and Cox proportional risk regression analyses were used for further analysis. Additionally, receiver operating characteristic curve and survival curves of subjects were plotted. The Pearson's χ^2 test showed that the prognosis of melanoma patients was significantly correlated with age, tumor grade, and decreased SEMA4D expression. Additionally, Spearman's correlation coefficient analysis showed that age, tumor grade, and SEMA4D expression were significantly correlated with prognosis. Univariate logistic regression analysis showed that age and tumor grade, and SEMA4D expression, were significantly correlated with prognosis. Older patients, a higher tumor grade, and lower SEMA4D expression were associated with a poorer prognosis. Multivariate logistic regression analysis showed that older patients had a poorer prognosis, and patients with lower SEMA4D expression levels had a significantly worse prognosis than patients with higher SEMA4D expression levels. Kaplan-Meier analysis showed that the survival time of older patients was lower than that of the younger patients. The survival times of patients with lower SEMA4D expression levels were significantly lower than that of patients with higher SEMA4D expression levels. Multivariate Cox regression analysis showed that the survival time of older patients was lower than that of younger patients. The survival time of melanoma patients with low SEMA4D expression was significantly lower than that of patients with higher SEMA4D expression. SEMA4D was significantly associated with melanoma, and lower SEMA4D expression was associated with a poorer survival prognosis in melanoma patients.

Correspondence to: Dr Zhi-Lei Zhang, Department of Hepatobiliary Surgery, The Fourth Affiliated Hospital of Hebei Medical University, 12 Chang'an District Health Road, Shijiazhuang, Hebei 050011, P.R. China
E-mail: zhilei3652@163.com

Key words: melanoma, semaphorin 4D, prognosis, survival time

Introduction

Melanoma is a highly malignant tumor that originates from melanocytes. It occurs primarily in the skin, mucous membranes, and viscera (1). Malignant melanomas may arise from congenital or acquired benign melanocytic nevi, malevolent dysplastic nevi, or may even develop *de novo* (2). For the last 5 years, the incidence and mortality rates of melanoma have increased on an annual basis, and the lethal age of melanoma is lower than that of other solid tumors (3,4). Melanoma can occur in all individuals of all ages, occurring more often in men, and the mortality rate of male patients is higher than that of females (5). Melanoma is stratified as follows: Grade I, tumor cells are confined to the epidermis above the basement membrane; Grade II, tumor cells have broken through the basement membrane and have invaded the dermal papillary layer; Grade III, the tumor cells fill the papillary layer of the dermis and invade further downwards, but have not reached the reticular layer of the dermis; Grade IV, the tumor cells have invaded the dermal reticular layer; and Grade V, the tumor cells have passed through the dermal reticular layer and invaded the subcutaneous fat layer (6). There are generally no obvious symptoms of occurrence during the earlier stages. During the later stages, ulcerations, impaired healing, regional or distant lymph node enlargement, and distant metastasis are observed (7). In addition to early surgical resection, melanoma lacks specific treatment options, with a high degree of malignancy, metastasis, and a poor prognosis (8,9). However, the cause of melanoma is not fully understood. It is generally hypothesized that several factors, such as race and genetics, trauma and stimulation, sunlight, and immunity, amongst others are all involved.

Bioinformatics can be used to study biological problems using the methods of applied mathematics, informatics, statistics, and computer science (10). The research materials and results of bioinformatics analyses cover numerous types of biological data. The methods typically involve sequence alignment, gene recognition, gene recombination, protein structure prediction, gene expression, protein response prediction, and evolutionary modeling (11).

Semaphorin 4D (SEMA4D) is a member of the Semaphorin family of axon-directed molecules, also known as CD100, hypothesized initially to be axon-directed factors affecting neural development (12). In addition to regulating axonal orientation, angiogenesis, and tumor metastasis, SEMA4D also plays an essential role in the immune system. The Gene Ontology (GO) annotations related to SEMA4D include signal receptor binding and transmembrane signal receptor activity. An important paralog of this gene is SEMA4B, which was discovered as a negative regulator of the PI3K/AKT signaling pathway in breast cancer (13). However, the relationship between SEMA4D and melanoma is unclear.

In this study, bioinformatics analysis was used to verify the potential role of SEMA4D in melanoma, and 272 melanoma patients were recruited to study the impact of abnormal expression of SEMA4D on the prognosis and survival time of melanoma patients, with the aim of identifying the molecular mechanism involved.

Materials and methods

Expression of SEMA4D in a database. Gene Expression Profiling Interactive Analysis (GEPIA; http://gepia.cancer-pku.cn/) was used to analyze the expression of SEMA4D in melanoma tumors. GEPIA generates box plots for comparing expression in several types of cancer. The method used for differential analysis is independent-samples t-test when two groups were compared, using the disease state (Tumor or Normal) as the variable for calculating differential expression.

Patients. A total of 272 patients diagnosed with melanoma and liver cancer at the Fourth Hospital of Hebei Medical University were selected for inclusion between March 2015 and June 2020.

The inclusion criteria were: Aged 18-80 years old, diagnosed with melanoma, with normal cardiopulmonary function, and normal coagulation.

The exclusion criteria were: Aged <18 years or >80 years, required emergency surgery, or the patient and/or their family did not agree to participate in the trial.

The Ethics Committee of the Fourth Hospital of Hebei Medical University approved the present study (approval no. FHBM2015014), and all patients signed informed consent.

Parameters assessed. Based on the clinical data, patients were classified by sex (male/female), age (≤60/>60), tumor size (≤3cm/>3cm), family history of cancer (no/yes), tumor grade (low/high), SEMA4D expression (Low/High), tumor stage (low/high), and prognosis (survival ≥30 months/survival <30 months). Patients' survival times were recorded during follow-up. Patients were classified according to Breslow depth (low/high), radial vs. vertical growth phase (early/late), presence of ulceration (no/yes), tumor-infiltrating lymphocyte numbers (low/high), lymphovascular spread (no/yes), regression (no/yes), metastases plus (no/yes), disease stage (mild/severe), discus melanin (low/high) and SEMA3B expression (low/high).

RNA extraction. An RNA Extraction Kit (cat. no. G3013; Wuhan Servicebio Technology Co., Ltd.) was used according to the manufacturer's protocol. Pre-sample treatment: 1 ml whole blood was taken, centrifuged at 80,000 x g for 5 min at 4˚C, and the supernatant was discarded. To the pellet, 3 ml erythrocyte lysate reagent (cat. no. R1010; Beijing Solarbio Science & Technology Co., Ltd.) was added, mixed, and placed at 4˚C (or room temperature) for 10 min, centrifuged again at 80,000 x g for 5 min at 4˚C, and the supernatant was discarded. Subsequently, red blood cell lysate (1 ml; cat. no. R1010; Beijing Solarbio Science & Technology Co., Ltd.) was added 1-2 times until the liquid was clear, and then the precipitate was collected via centrifugation (80,000 x g for 5 min at 4˚C). Next, 1 ml RNA extract (cat. no. G3013; Wuhan Servicebio Technology Co., Ltd.) was added and mixed well by shaking. After pre-treatment, the supernatant was centrifuged at 100,000 x g for 10 min at 4˚C. Trichloromethane (250 μl) was added, after which the tube was turned upside down for 15 sec, mixed thoroughly, left to stand for 3 min, centrifuged at 100,000 x g at 4˚C for 10 min, and 400 μl of the supernatant was transferred into a new centrifuge tube. To this, isopropyl alcohol (equivalent to 80% of the volume in the tube) was added and mixed thoroughly., after which it was incubated at -20˚C for 15 min. After centrifugation at 100,000 x g at 4˚C for 10 min, the white precipitate at the bottom of the tube was the desired RNA. The supernatant was removed and 1.5 ml 75% ethanol was added to wash the precipitate. The mixture was again centrifuged at 100,000 x g at 4˚C for 5 min, after which the supernatant was obtained. The centrifuge tube was placed on an ultra-clean platform for drying for 3 min, after which 15 μl RNA solvent (cat. no. XY-TE-0129; Shanghai Xuanya Biotechnology Co., Ltd.). This solution was incubated at 55˚C for 5 min. A Nanodrop 2000 was used to measure RNA concentration and purity. The expression levels of the related genes were detected by reverse transcription-quantitative (RT-q)PCR.

RT-qPCR. Total RNA was extracted from the blood samples using TRIzol® reagent (Beijing Biolab Technology Co., Ltd.) and reverse transcribed into cDNA using a Servicebio® RT First Strand cDNA synthesis kit (cat. no. G3330, Wuhan Servicebio Biotechnology Co., Ltd.) for 60 min at 42˚C, terminating the reaction by heating at 70˚C for 5 min. qPCR was performed in a Light Cycler® 4800 System (Roche Diagnostics) with a specific set of primers for the amplification of select hub genes. The thermocycling conditions used were: 95˚C for 15 sec and 60˚C for 60 sec (a total of 30 cycles). The relative quantification units (relative quantification=$2^{-\Delta\Delta Cq}$, where Cq represents quantification cycle values) of each sample were calculated and presented as fold change of gene expression relative to the control group. GAPDH was used as the endogenous control. The sequences of the primers used were: SEMA4D forward, TGAGCCAGACATCTACAACTACT and reverse, GAGTGCGTTCACAGCGAAGA; and GAPDH forward,

Table I. Relevant characteristics of patients with melanoma.

Characteristic	n	Prognosis, n (%)		P-value
		Survival ≥30 months	Survival <30 months	
Sex				0.800
Male	135	61 (22.4)	74 (27.2)	
Female	137	64 (23.5)	73 (26.8)	
Age, years				0.002[a]
≤60	99	58 (21.3)	41 (15.1)	
>60	173	67 (24.6)	106 (39.0)	
Tumor size, cm				0.620
≤3	137	65 (23.9)	72 (26.5)	
>3	135	60 (22.1)	75 (27.6)	
Family history				0.263
No	112	56 (20.6)	56 (20.6)	
Yes	160	69 (25.4)	91 (33.5)	
Tumor grade				0.007[a]
Low	139	75 (27.6)	64 (23.5)	
High	133	50 (18.4)	83 (30.5)	
Semaphorin 4D expression				<0.001[b]
Low	138	29 (10.7)	109 (40.1)	
High	134	96 (35.3)	38 (14.0)	
Tumor stage				0.592
Low	141	67 (24.6)	74 (27.2)	
High	131	58 (21.3)	73 (26.8)	

Pearson's χ^2. [a]P<0.01, [b]P<0.001.

TGAAGGTCGGAGTGAACGGAT and reverse, CGTTCT CAGCCTTGACCGTG.

Western blotting. Total protein from tissues was extracted and the concentration was determined using the UV method (14). Next, one-quarter of the protein sample (by volume) was added to 5x protein loading buffer (reduced), and boiled at 100˚C for 10 min. After cooling, the samples were aliquoted and stored at -80˚C until required. For western blotting, protein (4 μg) was loaded on 12% SDS-gels, resolved using SDS-PAGE, transferred to a PVDF membrane, blocked using 5% skimmed milk at room temperature for 1 h, and incubated with the primary antibody at 4˚C overnight. The following day, the membranes were washed with TBST three times (5 min/wash), incubated with the HRP-conjugated rabbit secondary antibody (1:5,000; cat. no. ab205718; Abcam) at room temperature for 1 h, and washed again as above. Signals were visualized using chemiluminescence reagent. The following antibodies were used: anti-Actin antibody (1:20,000; cat. no. 66009-1-Ig; ProteinTech Group, Inc.), anti-SEMA4D antibody (1:20,000; cat. no. 66582-1-Ig; ProteinTech Group, Inc.), anti-SEMA3B antibody (1:5,000; cat. no. ab48197; Abcam). Actin was used as the loading control.

Analysis of SEMA4D expression against survival in patients with melanoma. Kaplan-Meier (K-M) survival analysis, also known as Product-limit Estimate, is the most commonly used survival analysis method, which is primarily used to estimate the survival rate of patients and draw survival curves. A log-rank test was used to compare survival.

Statistical analysis. Data are presented as percentages. Pearson χ^2 and Spearman's rank correlation coefficient analysis were used to analyze clinical parameters and prognosis of melanoma patients. Univariate and multivariate logistic regression analyses were used to calculate odds ratios (ORs) of the prognostic variables in melanoma patients. Univariate and multivariate Cox proportional risk regression analyses were conducted to investigate the correlation between the melanoma patients' survival time and related factors. The receiver operating characteristic (ROC) curves were obtained using MedCalc software (version 19.0.4; MedCalc Software Ltd.). All other statistical analyses were performed in SPSS version 21.0 (IBM Corp.). P<0.05 was considered to indicate a statistically significant difference.

Results

Analysis of SEMA4D expression between melanoma and normal tissues. SEMA4D expression level in melanoma was significantly lower than that in normal tissues in the GEPIA database (Fig. 1).

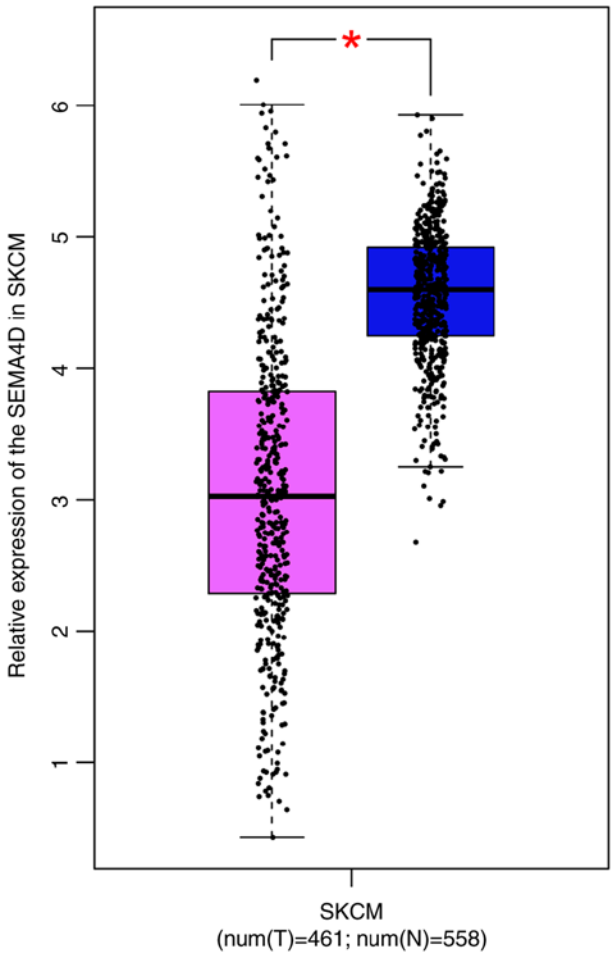

Figure 1. Comparison of SEMA4D expression between melanoma and normal tissues. *P<0.05. Purple, melanoma; Blue, normal. SEMA4D, semaphorin 4D. SKCM, skin cutaneous melanoma; T, tumor; N, normal.

Pearson χ^2 analysis for melanoma-related factors and patient prognosis. A Pearson χ^2 test was used to summarize the relationship between melanoma-related factors and patient prognosis. Age (P=0.002), tumor grade (P=0.007), and SEMA4D expression (P<0.001) were significantly associated with prognosis. However, sex (P=0.800), tumor size (P=0.620), family history (P=0.263), and tumor stage (P=0.592) were not significantly associated with prognosis (Table I).

Spearman's rank correlation coefficient analysis of melanoma-related factors and patient prognosis. Spearman's rank correlation coefficient showed that Age (ρ=0.192, P=0.001), tumor grade (ρ=0.164, P=0.007), SEMA4D (ρ=-0.508, P<0.001) were significantly correlated with prognosis. However, sex (ρ=-0.015, P=0.801), tumor size (ρ=0.030, P=0.621), family history (ρ=0.068, P=0.264) and tumor stage (ρ=0.033, P=0.593) had no significant correlation with prognosis (Table II).

Univariate logistic regression analysis of prognosis and related factors in melanoma patients. Univariate logistic regression analysis was used to determine the relationship between melanoma-related parameters and prognosis, OR, and 95% confidence intervals (95% CI). Table III shows the OR and 95% CI values of the subjects at the univariate

Table II. Relationship between the characteristics of patients with melanoma and classification of the patients' prognosis.

	Prognostic value	
Characteristics	ρ	P-value
Sex	-0.015	0.801
Age	0.192	0.001[b]
Tumor size	0.030	0.621
Family history	0.068	0.264
Tumor grade	0.164	0.007[a]
Semaphorin 4D	-0.508	<0.001[b]
Tumor stage	0.033	0.593

Spearman's rank correlation coefficient. [a]P<0.01, [b]P<0.001.

logistic regression level; the results show that age (OR=2.238, 95% CI: 1.353-3.703, P=0.003), tumor grade (OR=1.945, 95% CI: 1.199-3.157, P=0.005) and SEMA4D (OR=0.105, 95% CI: 0.060-0.184, P<0.001) were significantly associated with prognosis. The prognosis of older patients was worse than that of younger patients, the prognosis of patients with a high tumor grade was worse than that of patients with a lower tumor grade, and the prognosis of patients with low SEMA4D expression levels was significantly worse than that of patients with high SEMA4D levels. However, sex (OR=0.940, 95% CI: 0.584-1.515, P=0.0.791), tumor size (OR=1.128, 95% CI: 0.700-1.819, P=0.609), family history (OR=1.319, 95% CI: 0.812-2.142, P=0.279) and tumor stage (OR=1.140, 95% CI: 0.707-1.837, P=0.587) had no significant association with prognosis (Table III).

Multivariate logistic regression analysis of the association between melanoma characteristics and patient prognosis. Multivariate logistic regression was used to describe the OR and 95% CI of the subjects at the multivariate level. The results showed that the prognosis of older patients was worse than that of younger patients (OR=2.254, 95% CI: 1.230-4.133, P=0.009), and the prognosis of patients with low SEMA4D expression was significantly worse than that of patients with high SEMA4D expression levels (OR=0.106, 95% CI: 0.059-0.190, P<0.001). While sex (OR=0.652, 95% CI: 0.361-1.177, P=0.156), tumor size (OR=1.098, 95% CI: 0.622-1.939, P=0.746), family history (OR=1.200, 95% CI: 0.672-2.141, P=0.538), tumor grade (OR=1.677, 95% CI: 0.949-2.964, P=0.075) and tumor stage (OR=0.930, 95% CI: 0.524-1.650, P=0.805) had no significant association with prognosis (Table IV).

Univariate Cox regression analysis of the proportional risk of survival time in melanoma patients. Table V shows the univariate Cox regression hazard ratios (HRs) and 95% CI values for the melanoma patients. Older patients had lower survival times than younger patients (HR=1.894, 95% CI: 1.412-2.541, P<0.001), the survival time of melanoma patients with low SEMA4D expression levels was significantly lower than that of patients with high SEMA4D expression levels (HR=0.570, 95% CI: 0.431-0.755, P<0.001). While sex (HR=1.203, 95% CI: 0.914-1.583, P=0.188),

Table III. Association between melanoma-related parameters and prognosis based on univariate logistic regression analysis.

Characteristic	n	Prognosis		P-value
		Odds ratio	95% confidence interval	
Sex				0.791
Male	135	1	0.584-1.515	
Female	137	0.94		
Age, years				0.003[a]
≤60	99	1	1.353-3.703	
>60	173	2.238		
Tumor size, cm				0.609
≤3	137	1	0.700-1.819	
>3	135	1.128		
Family history				0.279
No	112	1	0.812-2.142	
Yes	160	1.319		
Tumor grade				0.005[a]
Low	139	1	1.199-3.157	
High	133	1.945		
Semaphorin 4D expression				<0.001[b]
Low	138	1	0.060-0.184	
High	134	0.105		
Tumor stage				0.587
Low	141	1	0.707-1.837	
High	131	1.14		

[a]P<0.01, [b]P<0.001.

Table IV. Relationship between melanoma-related parameters and patient prognosis by multivariate logistic regression analysis.

Characteristics	Prognostic value		
	Odds ratio	95% confidence interval	P-value
Sex	0.652	0.361-1.177	0.156
Age	2.254	1.230-4.133	0.009[a]
Tumor size	1.098	0.622-1.939	0.746
Family history	1.200	0.672-2.141	0.538
Tumor grade	1.677	0.949-2.964	0.075
Semaphorin 4D	0.106	0.059-0.190	<0.001[b]
Tumor stage	0.930	0.524-1.650	0.805

[a]P<0.01, [b]P<0.001.

tumor size (HR=1.033, 95% CI: 0.787-1.355, P=0.817), family history (HR=0.931, 95% CI: 0.703-1.232, P=0.617), tumor grade (HR=1.291, 95% CI: 0.982-1.696, P=0.067) and tumor stage (HR=1.169, 95% CI: 0.889-1.538, P=0.264) were not significantly associated with survival time (Table V).

Multivariate Cox regression analysis of the proportional risk of survival time in melanoma patients. All factors were included in the Cox regression model to control for the influence of confounding factors. Multivariate Cox proportional regression analysis showed that the survival time of older patients was lower than that of younger patients (HR=1.778, 95% CI: 1.301-2.430, P<0.001), and the survival time of melanoma patients with low SEMA4D expression levels was significantly lower than that of patients with high SEMA4D expression levels (HR=0.641, 95% CI: 0.473-0.867, P=0.004). However, sex (HR=0.936, 95% CI: 0.693-1.265, P=0.669), tumor size (HR=1.050, 95% CI: 0.797-1.384, P=0.727), family history (HR=0.947, 95% CI: 0.714-1.256, P=0.705), tumor grade (HR=1.317, 95% CI: 0.997-1.738, P=0.052) and tumor stage (HR=1.150, 95% CI: 0.870-1.519, P=0.327) were not significantly associated with survival time (Table VI).

ROC curve. ROC curves indicated that SEMA4D was sensitive (80.95%) and specific (85.36%) in predicting melanoma and was associated with a higher risk of melanoma (area under the curve=0.800, P<0.05) (Figs. 2 and 3).

The ROC curve indicated that the combined influence of all patient-related factors (SEMA4D, tumor grade, tumor stage, age, family history, sex and tumor size) were sensitive (85.42%) and specific (89.75%) for the prediction of melanoma (area under the curve=0.832) (Fig. 4).

Table V. Univariate Cox regression analysis of melanoma-related characteristics on the survival time of patients.

		Survival time		
Characteristics	n	Hazard ratio	95% confidence interval	P-value
Sex				0.188
Male	135	1	0.914-1.583	
Female	137	1.203		
Age, years				<0.001[a]
≤60	99	1	1.412-2.541	
>60	173	1.894		
Tumor size, cm				0.817
≤3	137	1	0.787-1.355	
>3	135	1.033		
Family history				0.617
No	112	1	0.703-1.232	
Yes	160	0.931		
Tumor grade				0.067
Low	139	1	0.982-1.696	
High	133	1.291		
Semaphorin 4D expression				<0.001[a]
Low	138	1	0.431-0.755	
High	134	0.57		
Tumor stage				0.264
Low	141	1	0.889-1.538	
High	131	1.169		

[a]P<0.001.

Table VI. Influence of melanoma-related characteristics on patient survival time based on multivariate Cox regression analysis.

	Survival time		
Characteristics	Hazard ratio	95% confidence interval	P-value
Sex	0.936	0.693-1.265	0.669
Age	1.778	1.301-2.430	<0.001[b]
Tumor size	1.050	0.797-1.384	0.727
Family history	0.947	0.714-1.256	0.705
Tumor grade	1.317	0.997-1.738	0.052
Semaphorin 4D	0.641	0.473-0.867	0.004[a]
Tumor stage	1.150	0.870-1.519	0.327

[a]P<0.01, [b]P<0.001.

Survival analysis of factors associated with melanoma. Analysis of the patient survival curves showed that melanoma patients with lower SEMA4D expression had a shorter survival time compared with those with above median levels of SEMA4D expression levels (Fig. 5).

RT-qPCR and western blotting analysis of SEMA4D expression. Using RT-qPCR to compare SEMA4D expression in normal tissues and melanoma tissues, it was shown that the expression levels of SEMA4D were significantly lower in melanoma tissues (Fig. 6A). Furthermore, the results of western blotting confirmed these results at the protein level. That is, SEMA4D protein expression was downregulated in the melanoma tissues compared with the normal tissues. There was no significant difference in the expression of SEMA3B between the melanoma tissues and normal tissues (Fig. 6B).

Discussion

In this study, it was shown that the prognosis of melanoma patients was significantly correlated with age, tumor grade, and SEMA4D expression. Spearman correlation coefficient analysis showed that age, tumor grade, and SEMA4D expression were significantly correlated with prognosis. Univariate logistic regression analysis showed that age and tumor grade, and SEMA4D expression were significantly correlated with prognosis. Older patients, a higher tumor grade, and lower SEMA4D expression levels were associated with a poor prognosis. Multivariate logistic regression analysis showed that older patients had a poorer prognosis, and patients with low SEMA4D expression had a significantly worse prognosis than patients with high SEMA4D expression. Univariate Cox

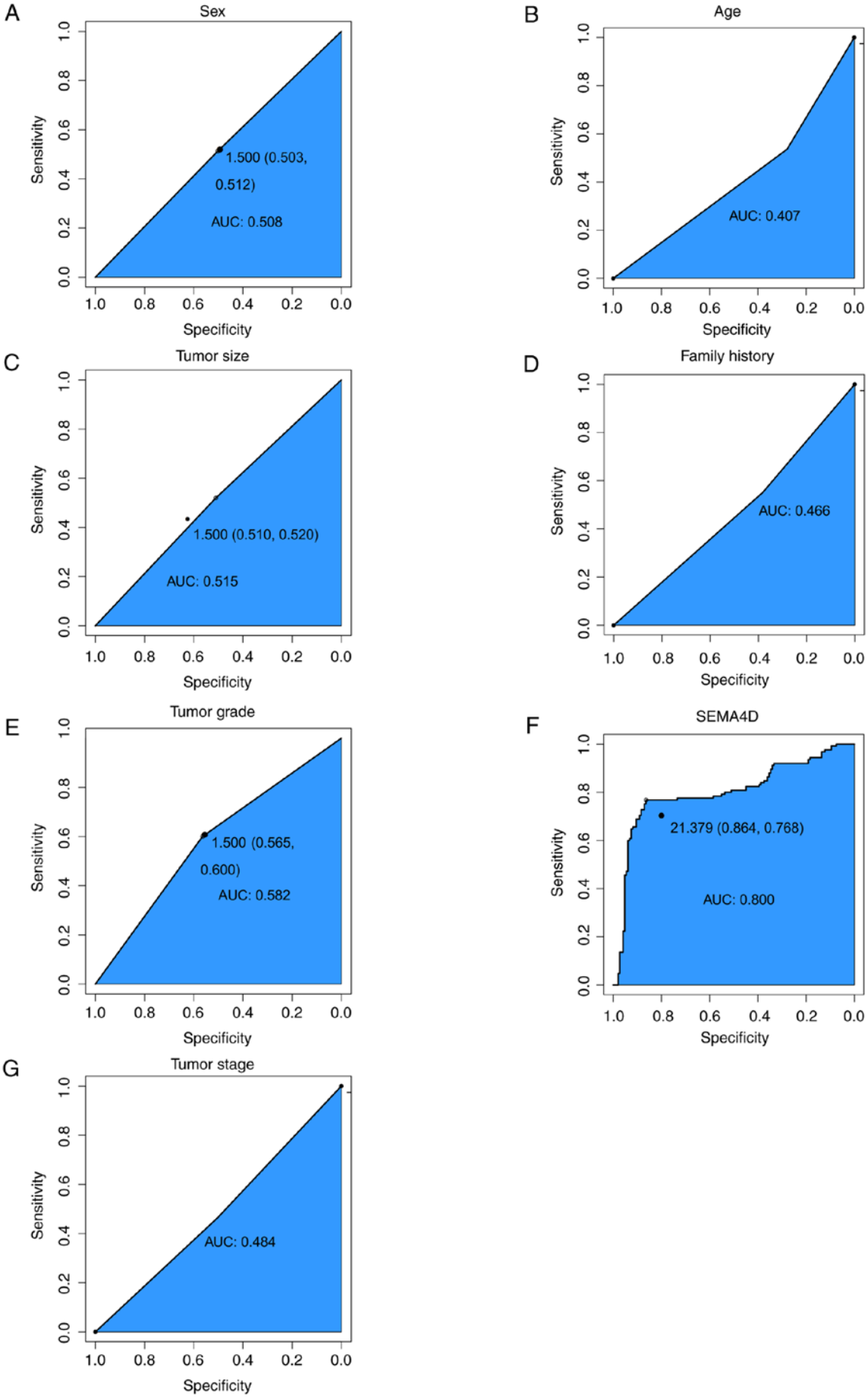

Figure 2. ROC curves. ROC curves of (A) Sex, (B) Age, (C) Tumor size, (D) Family history, (E) Tumor grade, (F) SEMA4D, and (G) Tumor stage. SEMA4D, semaphorin 4D; ROC, receiver operating characteristic; AUC, area under the curve.

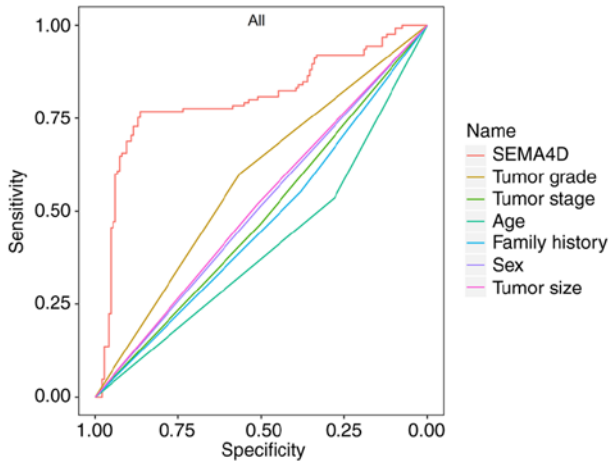

Figure 3. Receiver operating characteristic curve of all relevant factors for melanoma. SEMA4D, semaphorin 4D.

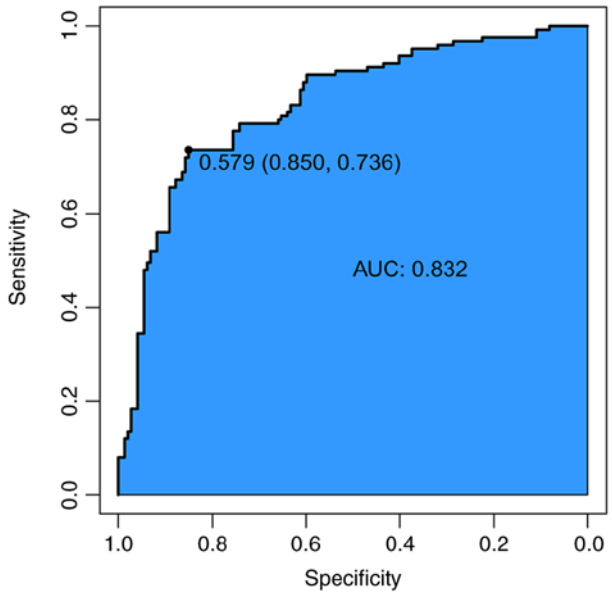

Figure 4. Receiver operating characteristic curve of the combined influence of all patient-related factors on melanoma. AUC, area under the curve.

regression analysis showed that the survival time of older patients was lower than that of younger patients, and the survival time of patients with low SEMA4D expression was significantly lower than that of patients with high SEMA4D expression. Multivariate Cox regression analysis showed that the survival time of older patients was lower than that of younger patients, and the survival time of melanoma patients with low SEMA4D expression was significantly lower than that of patients with high SEMA4D expression.

Melanoma is the most aggressive and deadly form of skin cancer (15). The production of a variety of pro-inflammatory cytokines secreted by macrophages, T lymphocytes and B lymphocytes in the tumor microenvironment encourages the survival and growth of tumor cells (16). Studies have shown that melanoma often has noticeable inflammatory reactions in the histopathological examination, and skin inflammation significantly promotes the growth of melanoma (17-19). Inflammation is a potential biomarker for stratified immunotherapy and targeted therapy in patients with melanoma (20). In the inflammatory tumor microenvironment of melanoma, immune cells, extracellular matrix proteins, cytokines, and other factors affect the progression of melanoma (21,22). Melanoma-related inflammation involves multiple regulatory pathways (20), and inflammation and immune response are critical to developing and treating melanoma.

SEMA4D is a protein-coding gene that is physiologically expressed on the surfaces of T cells, activated B cells, mature dendritic cells, macrophages, neutrophils, and natural killer cells (23). SEMA4D is involved in several processes, including positive regulation of phosphatidylinositol three kinase signaling, neuronal projection development, and regulation of phosphate metabolism. SEMA4D plays a crucial role in axonal orientation in the nervous system, activation of T and B cells in the immune system, and regulation through various signal transduction pathways (24).

SEMA4D is the first signaling element to play a role in the immune system (25). It exists in a soluble functional form that can bind to multiple receptors involved in immune regulation and inflammatory responses (26). SEMA4D shows varying effects on the inflammatory phenotype of different cell types (27,28). The SEMA4D protein is a transmembrane protein expressed on T cells, and platelets, amongst other cells. Activating T cells and platelets results in the cleavage and release of active soluble fragments of SEMA4D during the activation process, and it may also be present in its soluble form following proteolytic cleavage during cell activation (29). SEMA4D promotes pro-inflammatory cytokine production in various cells by binding to its plexin receptor (30). Cholangitis, primary sclerosis, and autoimmune vasculitis are diseases connected to SEMA4D via pathways including nervous system development and semaphore connections (31). SEMA4D has also been reported to induce proinflammatory cytokine production and is involved in endothelial inflammation and vascular dysfunction (32). SEMA4D is involved in platelet and neutrophil activation, angiogenesis, and cancer metastasis (33,34). Other studies have shown that SEMA4D promotes bladder cancer proliferation and metastasis by activating the PI3K/AKT pathway (35).

SEMA4D is inextricably linked to inflammation. Thus, when SEMA4D is abnormally expressed, it induces an inflammatory response, which is typically associated with the development and progression of cancer and is one of the initiation processes by which cells enter the tumor microenvironment through specific cytokines called chemokines (36). Inflammation also plays a decisive role in the initiation, promotion, malignant transformation, invasion, and metastasis of tumor development (37). Several cancers form at a site of infection, chronic irritation, and inflammation, and inflammatory cells have a powerful influence on tumor development (38). Inflammatory cells and the chemokines and cytokines they produce affect the entire tumor organ and regulate growth, migration, and differentiation of all cell types in the tumor microenvironment, including tumor cells, fibroblasts, and endothelial cells (39,40). Therefore, SEMA4D expression levels may play a role in the occurrence and development of melanoma through an inflammatory response.

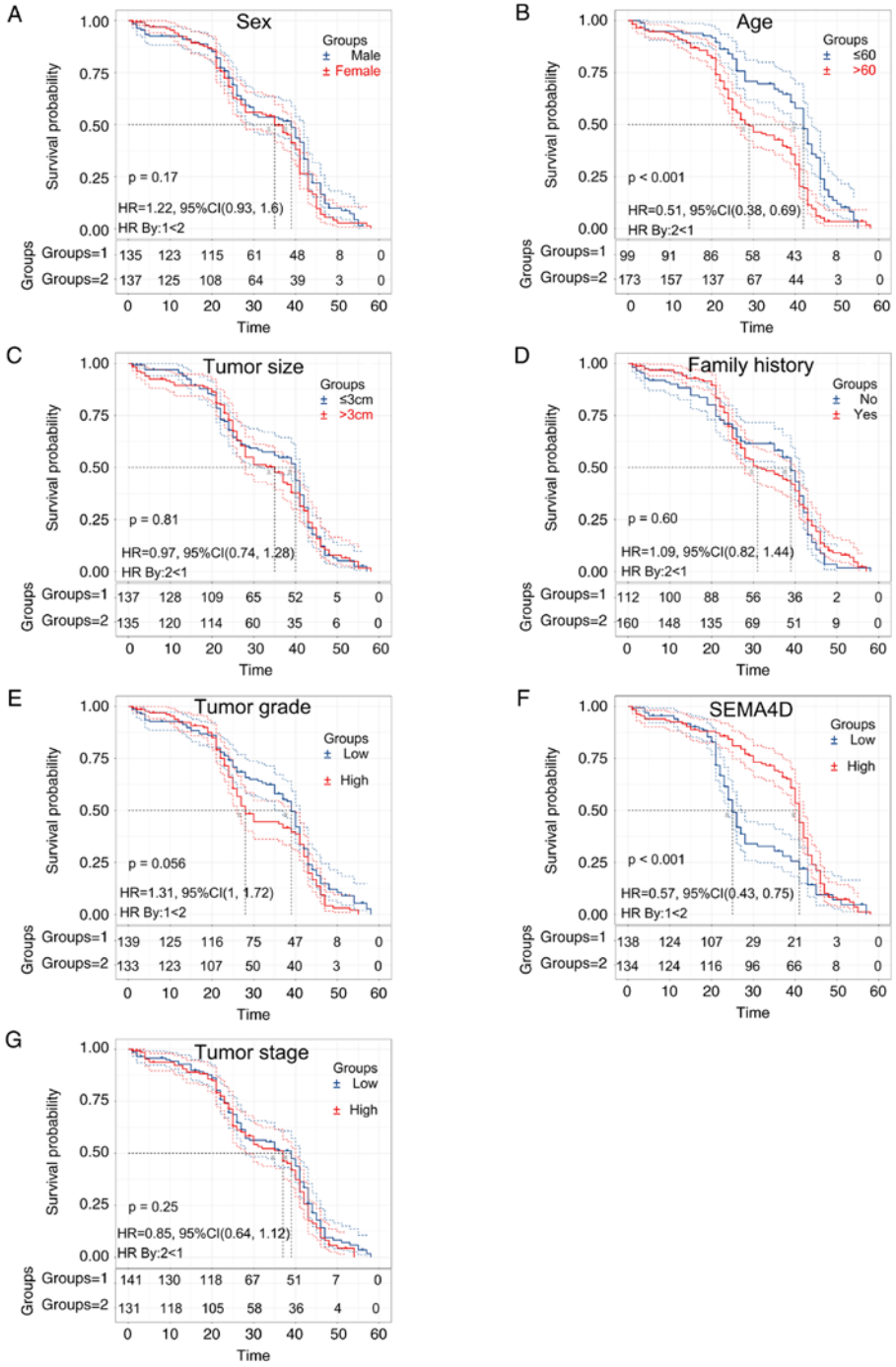

Figure 5. Survival curve analysis. Survival curve based on (A) Sex, (B) Age, (C) Tumor size, (D) Family history, (E) Tumor grade, (F) SEMA4D, and (G) Tumor stage. SEMA4D, semaphorin 4D; HR, hazard ratio; CI, confidence interval.

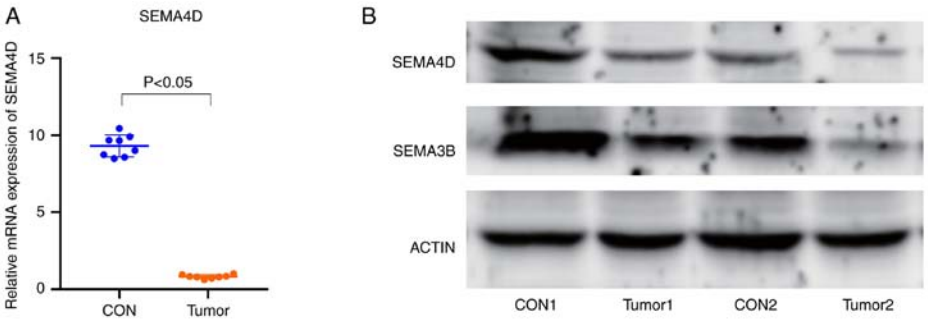

Figure 6. Expression of SEMA4D in liver cancer tissues. (A) mRNA expression of SEMA4D. (B) Protein expression levels of SEMA4D and SEMA3B. SEMA, semaphoring; CON, control.

The present study has some limitations. Although clinical data have been examined and analyzed, the molecular mechanisms by which SEMA4D expression levels affect melanoma prognosis and survival have not been validated in animal models. Therefore, future studies should focus on animal experiments to explore the molecular pathway and mechanism of SEMA4D in melanoma.

In conclusion, SEMA4D expression levels were shown to be significantly correlated with the prognosis and survival time of melanoma patients. Low SEMA4D expression is associated with a poorer prognosis and reduced survival times in melanoma patients. As a potential molecular marker of poor survival and prognosis of melanoma, the low expression of SEMA4D provides a novel direction for identifying the molecular mechanism underlying the development and progression of melanoma.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Authors' contributions

XL and ZZ conceived and designed the study. CZ, WY, RW and YZ participated in data analysis and interpretation. WY and SL completed the experiments. SL and RW drafted and revised key theories in the paper. YZ and ZZ answered academic questions. ZZ made substantial contributions to the conception and design of the study. ZZ and WY confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The Ethics Committee of the Fourth Hospital of Hebei Medical University approved the present study (approval no. FHBM2015014), and all patients signed informed consent.

Patient consent for publication

All patients and their families were informed in writing and consented to publication of this study.

Competing interests

The authors declare that they have no competing interests.

References

1. Dzwierzynski WW: Melanoma risk factors and prevention. Clin Plast Surg 48: 543-550, 2021.
2. Ahmed B, Qadir MI and Ghafoor S: Malignant Melanoma: Skin cancer-diagnosis, prevention, and treatment. Crit Rev Eukaryot Gene Expr 30: 291-297, 2020.
3. Bruno W, Dalmasso B, Barile M, Andreotti V, Elefanti L, Colombino M, Vanni I, Allavena E, Barbero F, Passoni E, *et al*: Predictors of germline status for hereditary melanoma: 5 years of multi-gene panel testing within the Italian Melanoma Intergroup. ESMO Open 7: 100525, 2022.
4. Ismail H, Helby J, Hölmich LR, H Chakera A, Bastholt L, Klyver H, Sjøgren P, Schmidt H, Schöllhammer L, Nordestgaard BG and Bojesen SE: Genetic predisposition to long telomeres is associated with increased mortality after melanoma: A study of 2101 melanoma patients from hospital clinics and the general population. Pigment Cell Melanoma Res 34: 946-954, 2021.
5. Guo W, Wang H and Li C: Signal pathways of melanoma and targeted therapy. Signal Transduct Target Ther 6: 424, 2021.
6. Craig S and Virós A: New biomarkers improve stratification of patients with melanoma. Br J Dermatol 182: 5-6, 2020.
7. Sun L and Arbesman J: Canonical signaling pathways in melanoma. Clin Plast Surg 48: 551-560, 2021.
8. Phoon YP, Tannenbaum C and Diaz-Montero CM: Immunobiology of Melanoma. Clin Plast Surg 48: 561-576, 2021.
9. Skudalski L, Waldman R, Kerr PE and Grant-Kels JM: Melanoma: An update on systemic therapies. J Am Acad Dermatol 86: 515-524, 2022.
10. Chen C, Hou J, Tanner JJ and Cheng J: Bioinformatics methods for mass spectrometry-based proteomics data analysis. Int J Mol Sci 21: 2873, 2020.
11. Fu Y, Ling Z, Arabnia H and Deng Y: Current trend and development in bioinformatics research. BMC Bioinformatics 21 (Suppl 9): S538, 2020.
12. Lu Q, Cai P, Yu Y, Liu Z, Chen G and Zeng Z: Sema4D correlates with tumour immune infiltration and is a prognostic biomarker in bladder cancer, renal clear cell carcinoma, melanoma and thymoma. Autoimmunity 54: 294-302, 2021.
13. Wang X, Jian W, Luo Q and Fang L: CircSEMA4B inhibits the progression of breast cancer by encoding a novel protein SEMA4B-211aa and regulating AKT phosphorylation. Cell Death Dis 13: 794, 2022.
14. Hughes AJ and Herr AE: Microfluidic Western blotting. Proc Natl Acad Sci USA 109: 21450-21455, 2012.
15. Castañeda-Reyes ED, Perea-Flores MJ, Davila-Ortiz G, Lee Y and Gonzalez de Mejia E: Development, characterization and use of liposomes as amphipathic transporters of bioactive compounds for melanoma treatment and reduction of skin inflammation: A review. Int J Nanomedicine 15: 7627-7650, 2020.
16. Pereira J, Bessa C, Matos P and Jordan P: Pro-Inflammatory cytokines trigger the overexpression of tumour-related splice variant RAC1B in polarized colorectal cells. Cancers (Basel) 14: 1393, 2022.
17. Ohno F, Nakahara T, Kido-Nakahara M, Ito T, Nunomura S, Izuhara K and Furue M: Periostin links skin inflammation to melanoma progression in humans and mice. Int J Mol Sci 20: 169, 2019.
18. Rossi N, Lee KA, Bermudez MV, Visconti A, Thomas AM, Bolte LA, Björk JR, de Ruijter LK, Newton-Bishop J, Harland M, *et al*: Circulating inflammatory proteins associate with response to immune checkpoint inhibition therapy in patients with advanced melanoma. EBioMedicine 83: 104235, 2022.
19. Chen Y, Zhang Y, Chen S, Liu W, Lin Y, Zhang H and Yu F: Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) sensitize melanoma cells to MEK inhibition and inhibit metastasis and relapse by inducing degradation of AXL. Pigment Cell Melanoma Res 35: 238-251, 2022.
20. Karlsson MJ, Costa Svedman F, Tebani A, Kotol D, Höiom V, Fagerberg L, Edfors F, Uhlén M, Egyhazi Brage S and Maddalo G: Inflammation and apolipoproteins are potential biomarkers for stratification of cutaneous melanoma patients for immunotherapy and targeted therapy. Cancer Res 81: 2545-2555, 2021.
21. Kalaora S, Nagler A, Wargo JA and Samuels Y: Mechanisms of immune activation and regulation: Lessons from melanoma. Nat Rev Cancer 22: 195-207, 2022.
22. Zhang H, Chen Z, Zhang A, Gupte AA and Hamilton DJ: The role of calcium signaling in melanoma. Int J Mol Sci 23: 1010, 2022.
23. Rajabinejad M, Asadi G, Ranjbar S, Afshar Hezarkhani L, Salari F, Gorgin Karaji A and Rezaiemanesh A: Semaphorin 4A, 4C, and 4D: Function comparison in the autoimmunity, allergy, and cancer. Gene 746: 144637, 2020.

24. Xie J, Wang Z and Wang W: Semaphorin 4D induces an imbalance of Th17/Treg cells by activating the Aryl hydrocarbon receptor in ankylosing spondylitis. Front Immunol 11: 2151, 2020.
25. Liu Y, Zhang WS, Tang ZH, Ye DD, Su S, Zhang SM and Qiu J: Anti-inflammatory effects of the immobilization of SEMA4D on titanium surfaces in an endothelial cell/macrophage indirect coculture model. Biomed Mater 17: 015005, 2021.
26. Younis RH, Ghita I, Elnaggar M, Chaisuparat R, Theofilou VI, Dyalram D, Ord RA, Davila E, Tallon LJ, Papadimitriou JC, *et al*: Soluble Sema4D in plasma of head and neck squamous cell carcinoma patients is associated with underlying non-inflamed tumor profile. Front Immunol 12: 596646, 2021.
27. Maleki KT, Cornillet M and Björkström NK: Soluble SEMA4D/CD100: A novel immunoregulator in infectious and inflammatory diseases. Clin Immunol 163: 52-59, 2016.
28. Chapoval SP, Vadasz Z, Chapoval AI and Toubi E: Semaphorins 4A and 4D in chronic inflammatory diseases. Inflamm Res 66: 111-117, 2017.
29. Willner N, Goldberg Y, Schiff E and Vadasz Z: Semaphorin 4D levels in heart failure patients: A potential novel biomarker of acute heart failure. ESC Heart Fail 5: 603-609, 2018.
30. Movila A, Mawardi H, Nishimura K, Kiyama T, Egashira K, Kim JY, Villa A, Sasaki H, Woo SB and Kawai T: Possible pathogenic engagement of soluble Semaphorin 4D produced by γδT cells in medication-related osteonecrosis of the jaw (MRONJ). Biochem Biophys Res Commun 480: 42-47, 2016.
31. Yu Y, Zhou Y, Di C, Zhao C, Chen J, Su W, Wu Q, Wu M, Su X and Xia Z: Increased airway epithelial cell-derived exosomes activate macrophage-mediated allergic inflammation via CD100 shedding. J Cell Mol Med 25: 8850-8862, 2021.
32. Wu JH, Li YN, Chen AQ, Hong CD, Zhang CL, Wang HL, Zhou YF, Li PC, Wang Y, Mao L, *et al*: Inhibition of Sema4D/PlexinB1 signaling alleviates vascular dysfunction in diabetic retinopathy. EMBO Mol Med 12: e10154, 2020.
33. Lontos K, Adamik J, Tsagianni A, Galson DL, Chirgwin JM and Suvannasankha A: The role of semaphorin 4D in bone remodeling and cancer metastasis. Front Endocrinol (Lausanne) 9: 322, 2018.
34. Nishide M and Kumanogoh A: The role of semaphorins in immune responses and autoimmune rheumatic diseases. Nat Rev Rheumatol 14: 19-31, 2018.
35. Lu JJ, Su YW, Wang CJ, Li DF and Zhou L: Semaphorin 4D promotes the proliferation and metastasis of bladder cancer by activating the PI3K/AKT pathway. Tumori 105: 231-242, 2019.
36. Nagarsheth N, Wicha MS and Zou W: Chemokines in the cancer microenvironment and their relevance in cancer immunotherapy. Nat Rev Immunol 17: 559-572, 2017.
37. Singh N, Baby D, Rajguru JP, Patil PB, Thakkannavar SS and Pujari VB: Inflammation and cancer. Ann Afr Med 18: 121-126, 2019.
38. Schmitt M and Greten FR: The inflammatory pathogenesis of colorectal cancer. Nat Rev Immunol 21: 653-667, 2021.
39. Iyengar NM, Gucalp A, Dannenberg AJ and Hudis CA: Obesity and cancer mechanisms: Tumor microenvironment and inflammation. J Clin Oncol 34: 4270-4276, 2016.
40. Landskron G, De la Fuente M, Thuwajit P, Thuwajit C and Hermoso MA: Chronic inflammation and cytokines in the tumor microenvironment. J Immunol Res 2014: 149185, 2014.