Original Article

Bioinformatics analysis of prognostic value and immune cell infiltration of *SERPINA1* gene in cutaneous melanoma

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Background: Cutaneous melanoma (CM) has a poor overall prognosis. Immune checkpoint inhibitor (ICI) therapy effectively improves overall survival in individuals with advanced melanoma, but only some patients benefit. Serpin Family A Member 1 (*SERPINA1*), a type of proteinase inhibitor that is used for many targets, is abnormally expressed and plays a vital role in multiple cancers. However, little is known about the clinical significance of *SERPINA1* in CM.

Methods: The Cancer Genome Atlas (TCGA) and the gene expression omnibus (GEO) datasets were used to compare *SERPINA1* expression levels. The association between *SERPINA1* and other clinical factors were examined with R software, and receiver operating characteristic (ROC) curves for identification was developed. The Tumor IMmune Estimation Resource (TIMER) was used to examine the invasion of immune cells, markers for immune cells, and immunological checkpoints. The predictive value of *SERPINA1* DNA methylation levels for every CpG was analyzed with the MethSurv web tool. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were used to assess the roles of genes that interacted with *SERPINA1*. The Tumor Immune Dysfunction and Exclusion (TIDE) algorithm was used to predict *SERPINA1*'s response to ICIs.

Results: *SERPINA1* was substantially expressed in CM. Overexpression of *SERPINA1* was significantly associated with CM severity. The outcome for individuals with elevated *SERPINA1* expression was good (HR =0.54, P<0.001). Using *SERPINA1* expression levels, tumors and normal tissues could be reliably differentiated [area under the curve (AUC) =0.889]. Positive associations were found between *SERPINA1* in CM and the infiltration of immune cells and immunological checkpoints [programmed cell death-1 (PD-1) and CTLA-4]. The efficacy of immune checkpoint blockade (ICB) in patients with a low expression of *SERPINA1* was good. The GO pathway enrichment analysis showed that activation of neutrophil granulocytes participated in enrichment in the immune response pathway. Patients with low *SERPINA1* expression had low TIDE scores.

Conclusions: *SERPINA1* is involved not only in the development and progression of CM but also in the immunological control of CM. Thus, *SERPINA1* may serve as a possible biomarker for CM diagnosis, as well as its therapeutic target.

Keywords: Serpin Family A Member 1 (*SERPINA1*); cutaneous melanoma (CM); immune cells; immune checkpoints; methylation

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Introduction

Cutaneous melanoma (CM) is a malignant cancer derived from melanocytes in the skin which produce pigments and is characterized by high invasiveness and the ability to metastasize to distal organs (1). CM is one of the most serious skin cancers, and although early CM can be cured by local excision, the prognosis for CM patients with advanced metastatic CM remains poor (2). Blocking antibodies against programmed cell death-1 (PD-1), such as nivolumab, are generally effective in treating many malignant tumors. The efficacy of nivolumab in treating unresectable and metastatic CM is up to 40% (3) and it is effective for adjuvant treatment of surgically resectable and metastatic CM (4). However, not all patients benefit from PD-1 blockade (5). In addition, immune checkpoint inhibitors (ICIs) can cause adverse side effects related to immunity and are typically very expensive (6). Thus, defining biomarkers that can predict response to treatment is of great value in attempting to optimize the use of anti-PD-1/PD-L1 ICIs. It is well accepted that molecular biomarkers may facilitate the prognosis prediction for CM patients (7). Currently, there is no matured biomarkers is approved for HNSCC prognosis prediction. Therefore, it is expected and worth that the identification of novel biomarkers assisting with patient care and survival improvement.

The Serpin Family A Member 1 (*SERPINA1*) gene provides instructions for making α1-antitrypsin (AAT), a type of proteinase inhibitor that is used for many targets, such as serine proteinase. Previous research has shown that the expression of *SERPINA1* could be simulated via E2 in MCF-7 breast cancer cells, and the elevated expression of such protein suppressed colony formation (8). *SERPINA1* has been suggested as a marker for numerous disorders, including skin squamous cell carcinoma (9), hepatitis B (10), insulinoma (11), non-small cell lung cancer (NSCLC) (12), papillary thyroid carcinoma (PTC) (13), lung cancer (14) and breast cancer (15,16).

However, the expression and clinical value of *SERPINA1* in CM are unknown. The current research screened potential biomarkers by analyzing *SERPINA1* expression in CM and its relationship with clinical prognosis in an effort to improve treatment benefits for many patients. We present the following article in accordance with the TRIPOD reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-3873/rc).

Methods

Ethical statement

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Comparison of the SERPINA1 expression level

SERPINA1 expression levels in 33 human cancers, 470 CM tissues, and 1,809 samples collected from 54 tissue sites of nearly 1,000 individuals in the Genotype-Tissue Expression (GTEx) program were analyzed using data from The Cancer Genome Atlas (TCGA) dataset (https://portal.gdc.cancer.gov/). Additionally, *SERPINA1* expression was analyzed using the GSE100050 and GSE57715 gene expression profiles extracted from the Gene Expression Omnibus (GEO) dataset.

Correlation analysis of SERPINA1 and cancer stage and prognosis

The correlation of *SERPINA1* with tumor stage and prognosis was analyzed with the "ggplot2" R package. Using the survival software, a Kaplan-Meier (K-M) graph was constructed, and a log-rank test (L-RT) was conducted. The medical records included in this analysis were obtained from TCGA dataset.

Genetic alteration in patients with CM

The cBioPortal (www.cBioPortal.org) *SERPINA1* genomic map of CM patients was evaluated using three datasets derived from hepatocellular carcinoma of TCGA and Firehose Legacy: AMC Hepatology 2014, INSERM, and Nat. Genet. 2015. The K-M curve was plotted, and the survival curve was assessed using the L-RT. A P value <0.05 was indicative of a statistically significant difference.

DNA methylation information of SERPINA1

The methylation site of *SERPINA1*'s DNA in the TCGA database was investigated utilizing the MethSurv tool (https://biit.cs.ut.ee/methsurv/). Moreover, we also evaluated the predictive significance of CpG methylation in *SERPINA1* against overall survival (OS).

Correlation analysis of SERPINA1 with immune cell infiltration and immune checkpoints

The Tumor IMmune Estimation Resource (TIMER; https://cistrome.shinyapps.io/timer/) was used to evaluate the relationship between the expression of *SERPINA1* in filtration of immune cells and indicators of immune cells. In addition, we used TIMER and the R "ggplot2" program in conjunction with TCGA to assess the relationship between *SERPINA1* expression in CM and immunological checkpoints. Statistical significance was determined by a P value less than 0.05. The Tumor Immune Dysfunction and Exclusion (TIDE) algorithm was used to predict potential ICB responses.

Gene Ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses

The function of *SERPINA1* was explored by enriching GO terms and evaluating KEGG pathways with 20 positive and negative partner functional genes. Raw counts of RNA-sequencing information (level 3) and accompanying diagnostic data from CM were acquired from the TCGA database, the acquisition and application of which adhered to the applicable guidelines and rules.

Statistical methods

R (version 3.6.3) was used for the statistical evaluation. To make group comparisons, the Wilcoxon rank-sum test or Student's test (where applicable) was applied. Pearson or Spearman tests were used as appropriate to calculate the correlation coefficients. The K-M graph was plotted, and the survival curve was assessed using the L-RT. All statistical tests were two sided, with statistical significance set at $P \leq 0.05$.

Results

Pan-cancer analysis (PCA) for SERPINA1 expression

To investigate the potential function of *SERPINA1* in carcinogenesis, we included normal tissues as a control in the GTEx database and evaluated *SERPINA1* expression in 30 individual cancers. As demonstrated in *Figure 1A*, in comparison to normal tissue samples, the expression of *SERPINA1* was strongly up-regulated in 21 tumor types, including cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), colon adenocarcinoma (COAD), urothelial bladder carcinoma (BLCA), head and neck squamous cell carcinoma (HNSC), esophageal carcinoma (ESCA), invasive breast carcinoma (BRCA), glioblastoma multiforme (GBM), clear cell renal cell carcinoma (CCRCC), testicular germ cell tumors (TGCT), papillary renal cell carcinoma (PRCC), acute myeloid leukemia (LAML), low-grade brain glioma (LGG), pancreatic adenocarcinoma (PAAD), prostate adenocarcinoma (PRAD), ovarian serous cystadenocarcinoma (OV), rectum adenocarcinoma (READ), CM, stomach adenocarcinoma (STAD), thyroid carcinoma (THCA), uterine corpus endometrial carcinoma (UCEC), and uterine carcinosarcoma (UCS). *SERPINA1* was strongly downregulated in seven tumors, namely adrenocortical carcinoma (ACC), cholangiocarcinoma (CHOL), lymphoid neoplasm diffuse large B-cell lymphoma (DLBC), chromophobe renal cell carcinoma (CRCC), hepatocellular carcinoma (HC), squamous cell lung carcinoma (LUSC) and thymoma (THYM). However, there was no noticeable difference in *SERPINA1* expression in lung adenocarcinoma (LUAD), pheochromocytoma, or paraganglioma (PCPG). A high expression of *SERPINA1* in CM was also observed in the GSE100050 and GSE57715 datasets ($P<0.001$) (*Figure 1B,1C*).

SERPINA1 expression was associated with tumor stage and prognosis

SERPINA1 expression was significantly associated with T stage (*Figure 2A*), Breslow thickness (*Figure 2B*), and Clark level (*Figure 2C*). Patients with deeper infiltration had a lower *SERPINA1* expression. The K-M survival graph demonstrated that CM patients with high *SERPINA1* expression levels had favorable prognoses with better overall survival ($P<0.001$), Disease-specific survival ($P<0.001$), and Progression-free interval (PFI) ($P=0.016$) than those who had low *SERPINA1* expression levels (*Figures 3A-3C*). In addition, the univariate analysis indicated that aging, *SERPINA1*, and T-, N-, and M-stage were significantly correlated with OS ($P<0.05$) (*Table 1*, *Figure 3D*). Subsequent multivariate analysis showed that increased *SERPINA1* expression was a favorable and independently predictive variable (HR =0.850, $P=0.005$) (*Table 1*).

Value of SERPINA1 expression in diagnosis

As seen in the ROC curve for prognosis, *SERPINA1* expression accurately distinguished tumors from normal tissues (AUC =0.889) (*Figure 4*).

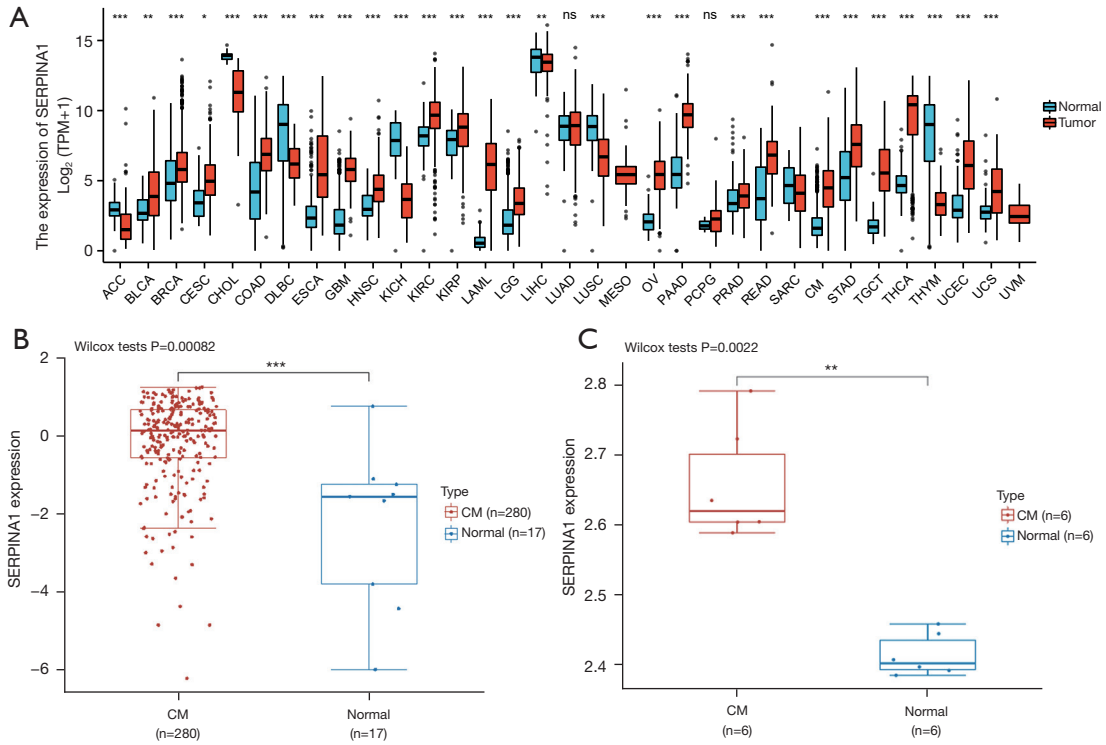


Figure 1 *SERPINA1* expression status in cancers. (A) Expression levels of *SERPINA1* in 33 kinds of tumor and normal tissues (comparing TCGA cancer findings with TCGA and GTEx normal findings), *, P<0.05; **, P<0.01; ***, P<0.001; ns, non-statistical variation. (B) Expression differences of *SERPINA1* in the. GSE100050 dataset. ***, P<0.001. (C) Expression differences of *SERPINA1* in the GSE57715 dataset. **, P<0.01. *SERPINA1*, Serpin Family A Member 1; CM, cutaneous melanoma; TCGA, The Cancer Genome Atlas; GTEx, Genotype-Tissue Expression.

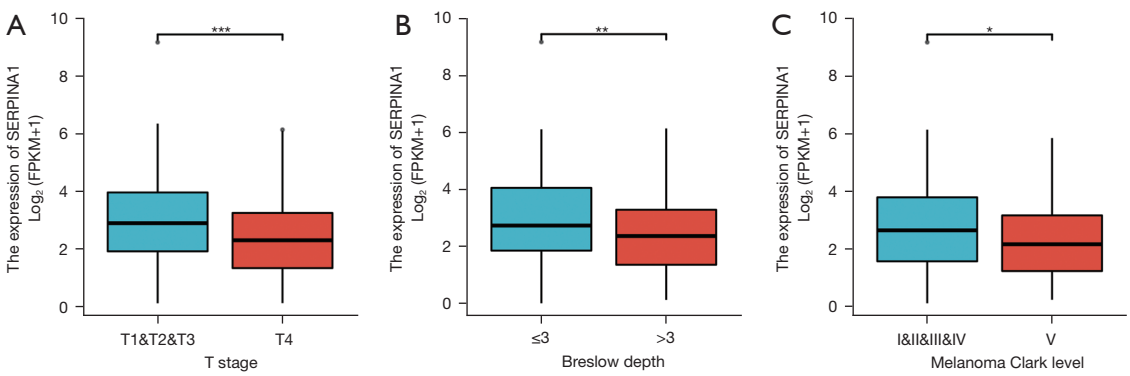
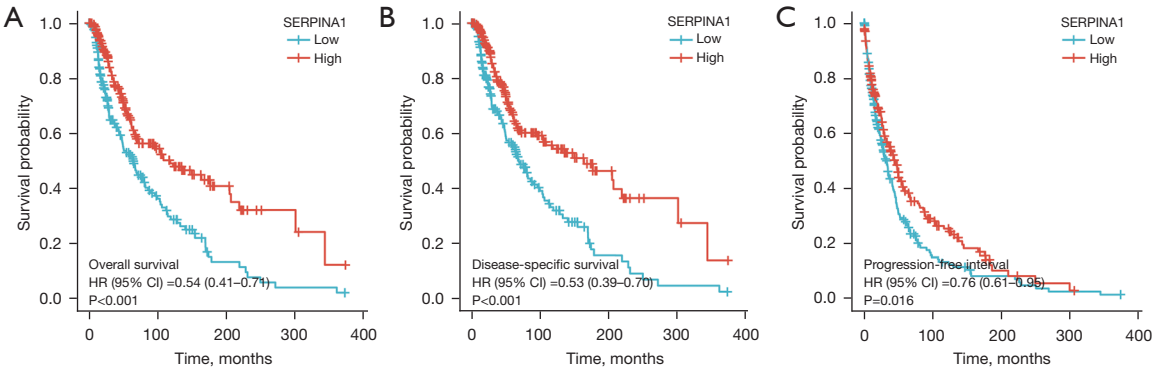


Figure 2 Relationship between the expression of *SERPINA1* and CM stage. The correlation between *SERPINA1* expression and T stage (A), Breslow depth (B), and melanoma Clark level (C). *, P<0.05; **, P<0.01; ***, P<0.001. *SERPINA1*, Serpin Family A Member 1; CM, cutaneous melanoma.

SERPINA1 genetic variation in CM individuals

A total of 1,165 individuals with CM were evaluated using three different databases: TCGA (cell 2015), MSKCC (clinical cancer research 2021), and DFCI (Science 2015). The proportion of *SERPINA1* genetic changes in CM was approximately 2.2% (*Figure 5A*). There was non-significant variation in OS (P=0.678) according to the K-M plots and L-RT (*Figure 5B*) among individuals with and without



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Characteristics	N	HR (95% CI)	P value
Gender	456		
Female	172	Reference	
Male	284	1.172 (0.879–1.563)	0.281
Age	456		
≤60	246	Reference	
>60	210	1.656 (1.251–2.192)	<0.001
T stage	361		
T1&T2&T3	208	Reference	
T4	153	2.290 (1.667–3.145)	<0.001
N stage	402		
N0&N1	297	Reference	
N2&N3	105	1.818 (1.310–2.523)	<0.001
M stage	430		
M0	406	Reference	
M1	24	1.897 (1.029–3.496)	0.04
SERPINA1	456	0.834 (0.761–0.914)	<0.001

Figure 3 *SERPINA1* expression and CM prognostic correlation. The comparison between the survival curves of (A) OS, (B) DSS, and (C) PFI for individuals with elevated (red) and reduced (blue) *SERPINA1* expression in CM. The P value threshold was 0.05. (D) Multivariate Cox analysis of *SERPINA1* expression. *SERPINA1*, Serpin Family A Member 1; CM, cutaneous melanoma; OS, overall survival; DSS, disease-specific survival; PFI, progression-free interval.

modifications.

MethSurv analysis of the methylation level of each single CpG in SERPINA1 DNA

The MethSurv analysis showed that there were 14 methylated CpG sites, of which DNAs in cg10832639 and cg16110645 were mostly methylated (*Figure 5C*). The methylation levels of 10 CpG sites associated with prognoses included cg02126235, cg02181506, cg05346611, cg09968361, cg10761141, cg10832639, cg16110645, cg20267408, cg24621042, and cg25042671 (P<0.05), as presented in *Table 2*. The OS of individuals with *SERPINA1* hypermethylation at two CpG sites, cg02181506 and cg20267408, was better than patients with low methylated CpGs, and the OS of patients with other high-methylated CpGs in *SERPINA1* was worse than patients with low methylated CpGs.

Correlation among SERPINA1 expression and infiltration of immune cells in CM

The K-M survival graph revealed that high *SERPINA1* expression was associated with a favorable prognosis; thus, we further explored the correlation between *SERPINA1* expression and infiltration of immune cells in CM. The relationship between *SERPINA1* expression and purity-regulated infiltration of immune cells (B cells, $CD4^+$ T cells, $CD8^+$ T cells, DCs, neutrophil granulocytes, and macrophages) was studied using TCGA and TIMER. Except for Mast cells, Tcm cells, and Tgd cells, our findings showed a positive relationship between *SERPINA1* expression and various ICI levels (*Figure 6A*). Our TIMER results also demonstrated a positive association between *SERPINA1* expression and the invasion of numerous immune cells (*Figure 6B*). The PCA also revealed that, in addition to CM, numerous other cancers expressed *SERPINA1*, including ACC, BLCA, BRCA, CESC, COAD,

Table 1 Univariate and multivariate analysis of CM

Characteristics	Total (N)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
Gender	456				
Female	172	Reference			
Male	284	1.172 (0.879–1.563)	0.281		
Age, years	456				
≤60	246	Reference			
>60	210	1.656 (1.251–2.192)	<0.001	1.234 (0.891–1.708)	0.206
T stage	361				
T1&T2&T3	208	Reference			
T4	153	2.290 (1.667–3.145)	<0.001	2.202 (1.575–3.079)	<0.001
N stage	402				
N0&N1	297	Reference			
N2&N3	105	1.818 (1.310–2.523)	<0.001	2.284 (1.555–3.356)	<0.001
M stage	430				
M0	406	Reference			
M1	24	1.897 (1.029–3.496)	0.040	1.920 (0.823–4.475)	0.131
SERPINA1	456	0.834 (0.761–0.914)	<0.001	0.850 (0.758–0.953)	0.005

CM, cutaneous melanoma; *SERPINA1*, Serpin Family A Member 1.

ESCA, GBM, HNSC, KICH, KIRC, KIRP, LGG, LUAD, LUSC, MESO, OV, PCPG, PRAD, SARC, TGCT, THCA, UCEC, and UCS and were positively associated with infiltration of different immune cells (*Figure 6C*).

SERPINA1 was positively correlated with markers for B cells (CD19 and CD38), markers for $CD8^+$ T cells (CD8A and CD8B), other T-cell subsets (Tfh, Th1, Th2, Th9, Th17, Th22, and Treg), biomarkers for M1 and M2 macrophages, and TAM, as seen in *Table 3*.

Relationship between SERPINA1 in CM expression and immunological checkpoints

SIGLEC15, TIGIT, CD274, HAVCR2, PDCD1, CTLA4, and LAG3 were IC-correlated transcripts, and considering the potential anti-cancer role of *SERPINA1* in CM, we extracted these seven genes and observed the gene expressions related to immune checkpoints. *SERPINA1* in CM was positively correlated with immune checkpoints (*Figure 7*).

Establishment of the gene network related to SERPINA1 in CM

To further understand which genes were possibly related to *SERPINA1* expression in TCGA-CM patients, a heatmap showing the first 20 genes positively or negatively associated with *SERPINA1* was generated (*Figure 8A*). Then, we conducted GO and KEGG enrichment analyses (*Figure 8B,8C*) to understand the potential biological functions of *SERPINA1* in CM. The GO analyses showed that the main biological process, neutrophil activation involved in immune response, might relate to biology relevant to *SERPINA1* (*Figure 8B*). The KEGG pathway analysis indicated that osteoclast differentiation was significantly enriched (*Figure 8C*).

Relationship between SERPINA1 in CM and immune checkpoint blockade (ICB)

The concept of tumor immunotherapy arose in the late 19th

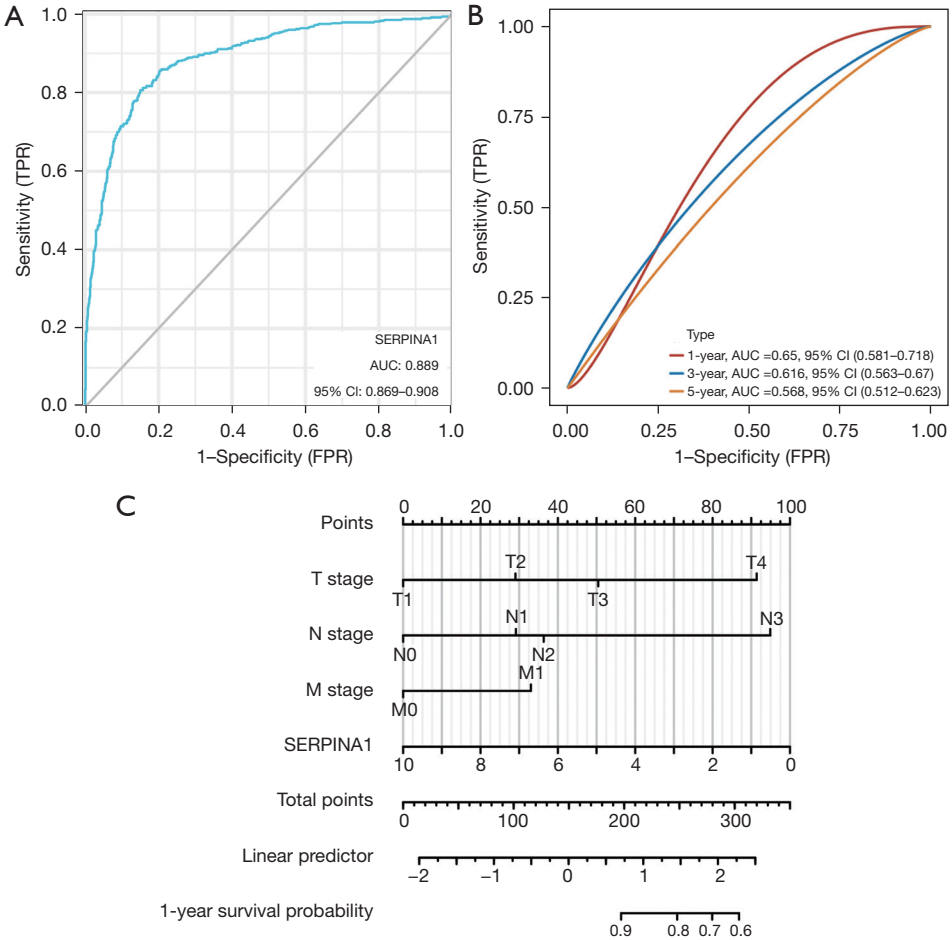

Figure 4 The diagnostic ROC curve for differentiating cancer versus normal tissue. ROC, receiver operating characteristic; TPR, true positive rate; FPR, false positive rate; AUC, area under the curve.

century and referred to a therapy that eliminated tumor cells using the body's own immune system. Treatment with ICB has thoroughly changed human cancer therapy. Based on expression profiles, the TIDE algorithm was designed to estimate the response of a single sample or some subtype to predict ICI responses (17). In the present study, TIDE utilized one set of gene expression biomarkers to analyze two distinct pathways of cancer immune escape: dysfunction of cytotoxic T lymphocytes (CTL) infiltrating the tumor and refusal of immune suppression elements against CTL. These results demonstrated that individuals with a low *SERPINA1* expression had a low TIDE score (*Figure 9*).

Discussion

Serpins are the largest and most widespread superfamily of protease inhibitors. The components of this superfamily, including AAT, ACT, C1 inhibitor, and antithrombin, have played (18,19). As a member of the serpin superfamily, *SERPINA1* is over-expressed in several cancerous tumors. Elevated *SERPINA1* expression was observed in PTC (20,21), breast cancer, prostate cancer, and colorectal adenocarcinoma (22-24), consistent with our results. Using the TCGA and GEO datasets and medical specimens, we validated the over-expression of *SERPINA1* in CM tissues. *SERPINA1* expression was negatively associated with T-stage, Breslow thickness, and Clark level. *SERPINA1* expression in CM and many malignancies was positively associated with ICI. Prognosis improved in line with a higher *SERPINA1*expression. *SERPINA1* expression was able to distinguish tumors from normal tissues and predicted overall survival at 1, 3, and 5 years, indicating that

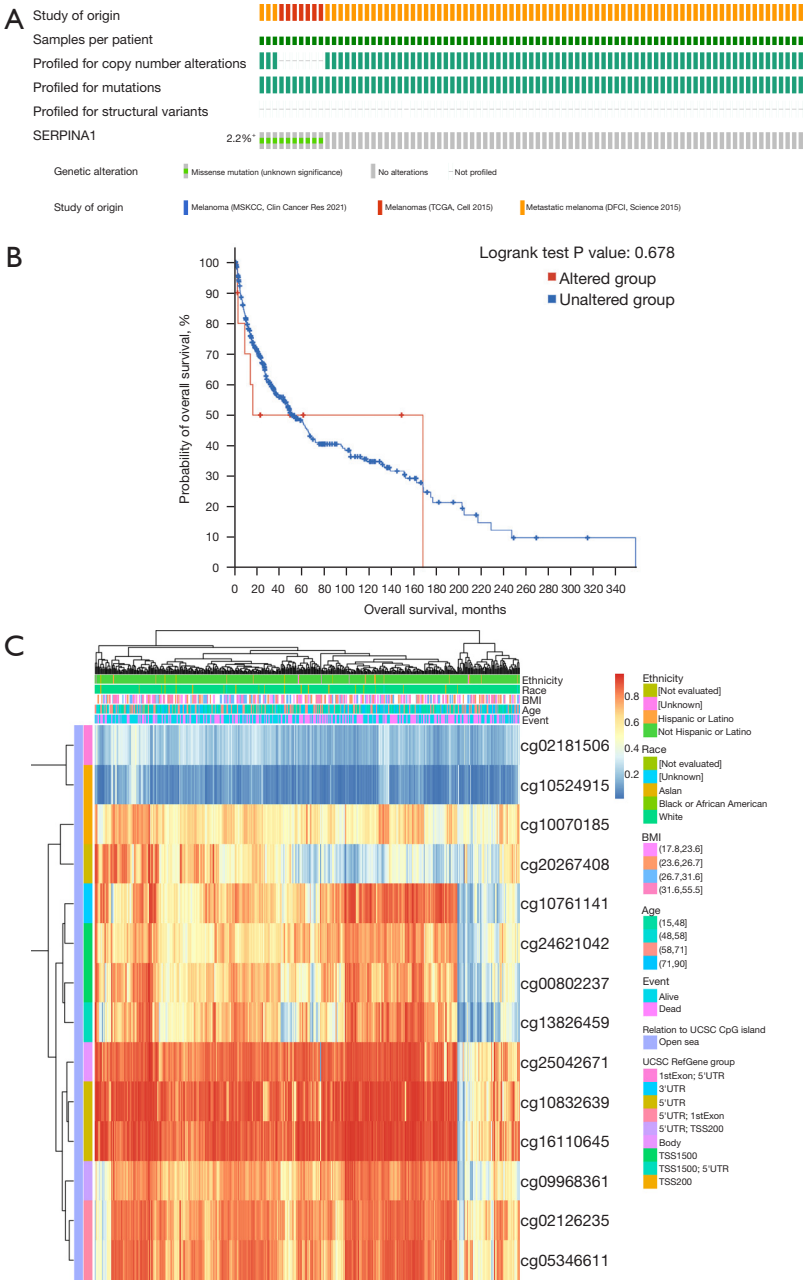

Figure 5 *SERPINA1* genetic change in CM. (A) OncoPrint graphic summary of *SERPINA1* mutation based on a query. (B) Graphs comparing OS between individuals with and without *SERPINA1* gene mutations. (C) Relationship among methylation degree and expression of *SERPINA1*. *SERPINA1*, Serpin Family A Member 1; CM, cutaneous melanoma; OS, overall survival; BMI, body mass index; UCSC, University of California Santa Cruz.

SERPINA1 might act as an effective and valuable biomarker in the diagnosis and prognosis of CM.

Although gene mutation is closely related to tumors and is always associated with poor prognosis, the mutation of the *SERPINA1* gene only exists in approximately 2.2% of CM cases, and there is no apparent correlation between gene mutation and poor OS. DNA methylation is a prevalent epigenetic pathway and exists in all tumor types.

Table 2 Effect of hypermethylation level on CM prognosis

Hypermethylation id	HR	95% CI	P
cg00802237	1.114	0.825–1.504	0.48
cg02126235	1.436	1.076–1.917	0.014
cg02181506	0.565	0.428–0.745	0.000052
cg05346611	1.391	1.012–1.912	0.042
cg09968361	1.411	1.038–1.917	0.028
cg10070185	0.786	0.569–1.085	0.14
cg10524915	0.742	0.534–1.03	0.074
cg10761141	1.607	1.208–2.138	0.0011
cg10832639	1.435	1.081–1.905	0.013
cg13826459	1.148	0.852–1.547	0.37
cg16110645	1.554	1.147–2.107	0.0045
cg20267408	0.559	0.42–0.745	0.00007
cg24621042	1.401	1.053–1.865	0.021
cg25042671	1.369	1.017–1.845	0.039

CM, cutaneous melanoma; HR, hazard ratio.

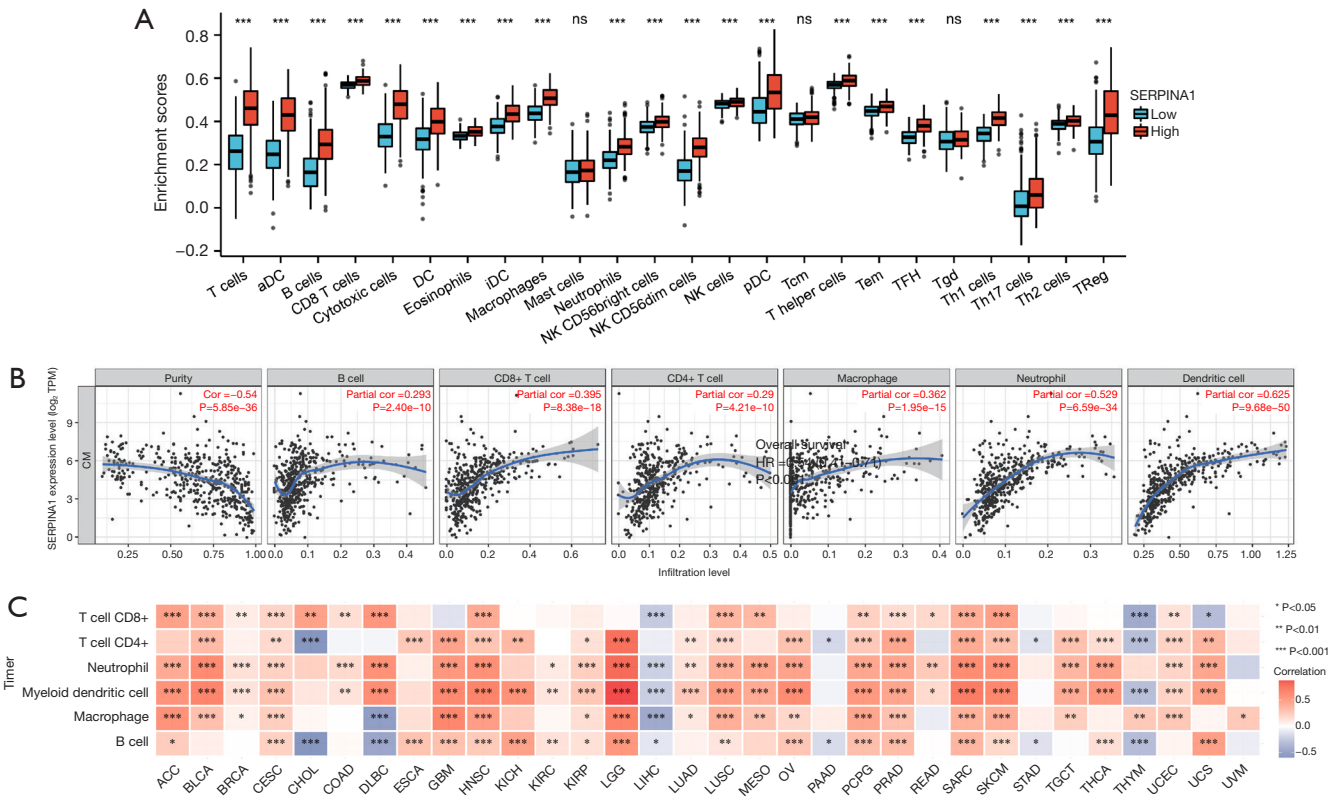

Figure 6 *SERPINA1* and tumor immune microenvironment association. *, P<0.05; **, P<0.01; ***, P<0.001. ns, non-statistical variation. (A) *SERPINA1* expression and ICI within CM. (B) TIMER database association of *SERPINA1* expression with immune infiltration level. (C) Multiple cancer tissues Spearman's association study heatmap of the immunological score and *SERPINA1* gene expression. *SERPINA1*, Serpin Family A Member 1; ICI, immune checkpoint inhibitor; CM, cutaneous melanoma; TIMER, Tumor IMmune Estimation Resource.

Table 3 Assessment of the relationship between *SERPINA1* expression and immune cell markers in CM

Immune cell	Biomarker	Cor	P value
B cell	CD19	0.556	***
	CD20 (KRT20)	−0.047	0.309
	CD38	0.699	***
CD8+ T cell	CD8A	0.710	***
	CD8B	0.713	***
Tfh	BCL6	0.320	***
	ICOS	0.616	***
	CXCR5	0.501	***
Th1	T-bet (TBX21)	0.724	***
	STAT1	0.536	***
	STAT4	0.574	***
	IL12RB2	0.043	0.348
	WSX1 (IL27RA)	0.238	***
	IFN-γ (IFNG)	0.668	***
	TNF-a (TNF)	0.616	***
Th2	CCR3	0.557	***
	GATA3	0.607	***
	STAT5A	0.214	***
	STAT6	0.005	0.919
Th9	IRF4	−0.110	*
	PU.1 (SPI1)	0.775	***
	TGFBR2	0.261	***
Th17	IL-17A	−0.008	0.866
	IL-21R	0.771	***
	IL-23R	0.235	***
	STAT3	0.324	***
Th22	AHR	0.073	0.112
	CCR10	0.282	***
Treg	CCR8	0.592	***
	CD25 (IL2RA)	0.631	***
	FOXP3	0.615	***
M1 macrophage	COX2 (PTGS2)	0.064	0.165
	INOS (NOS2)	0.047	0.309
	IRF5	0.606	***
M2 macrophage	ARG1	0.019	0.677
	CD206 (MRC1)	0.476	***
	CD115 (CSF1R)	0.701	***
TAM	PDCD1LG2	0.694	***
	CD80	0.683	***
	CD40	0.646	***
	TLR7	0.648	***
Natural killer cell	CD7	0.707	***
	KIR3DL1	0.512	***
	XCL1	0.593	***
Neutrophil	CD11b (ITGAM)	0.675	***
	CD15 (FUT4)	0.322	***
	CD66b (CEACAM8)	−0.024	0.596
Dendritic cell	CD1C	0.446	***
	CD11c (ITGAX)	0.654	***
	CD141 (THBD)	0.217	***

SERPINA1, Serpin Family A Member 1; CM, cutaneous melanoma. *, P<0.05, ***, P<0.001.

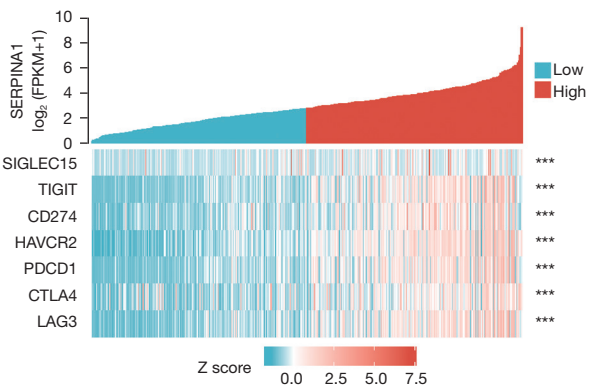

Figure 7 Immune checkpoint-related gene expression heatmap. ***, P<0.001.

We studied the association between DNA methylation rates and prognosis in CM patients. High methylation of eight CpG sites was related to poor OS, of which the degree of DNA methylation in cg10832639 and cg16110645 was highest.

Although immunotherapy improves the prognosis of CM

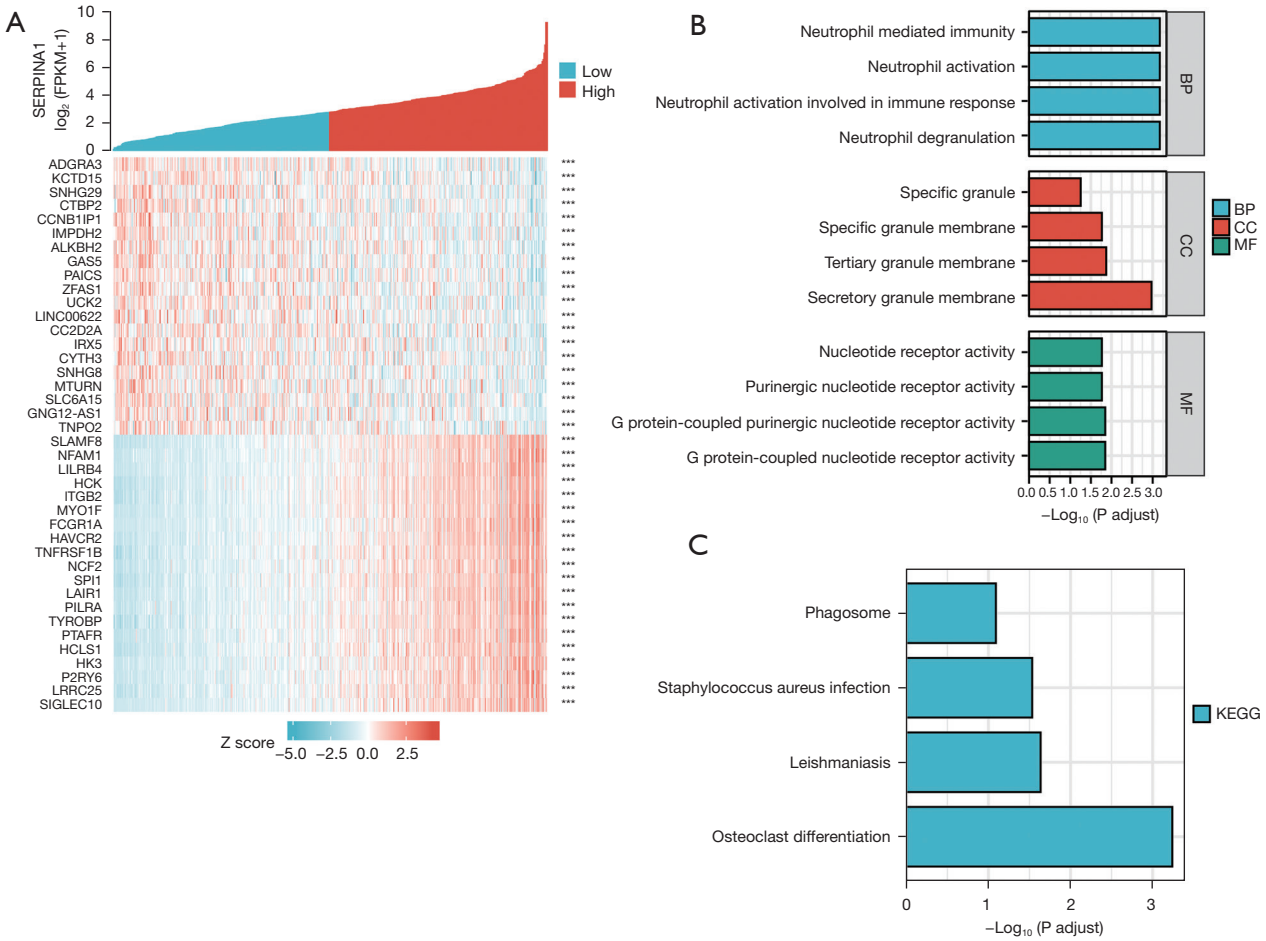

Figure 8 Establishment of the *SERPINA1*-related gene network in CM. (A) The heatmaps show the top 20 genes most highly correlated or anti-correlated with *SERPINA1* gene expression. (B) GO pathway enrichment analysis. (C) KEGG pathway enrichment analysis. ***, P<0.001. *SERPINA1*, Serpin Family A Member 1; CM, cutaneous melanoma; GO, Gene Ontology; BP, Biological Process; CC, Cellular Component; MF, Molecular Function; KEGG, Kyoto Encyclopedia of Genes and Genomes.

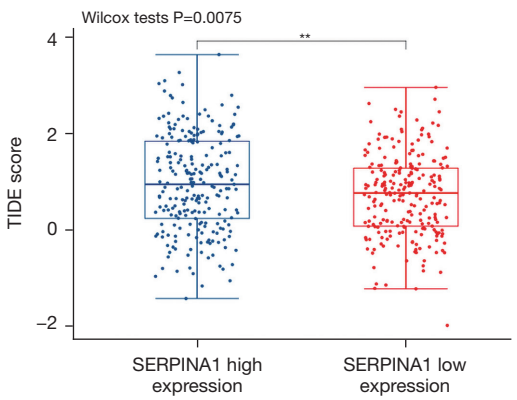

Figure 9 The distribution of immune response scores among various groups as indicated by the predicted findings. **, P<0.01. TIDE, Tumor Immune Dysfunction and Exclusion.

patients, from a value perspective, the optimal sequence and/or combination of immunotherapies are still unknown (25,26). Our study indicated that *SERPINA1* in CM was positively correlated with many immune cells and checkpoints. The PCA also found that, in addition to CM, many other tumors showed a positive correlation between *SERPINA1* and immune cells, which indicated that *SERPINA1* might control immune cells against CM via various immune cell populations. The TIDE algorithm showed that the TIDE score was low in patients with low *SERPINA1* expression. This suggests that individuals with low *SERPINA1* expression might benefit more from ICB and possibly achieve a longer OS following treatment with ICB.

The GO/KEGG analysis indicated that *SERPINA1* was

closely related to immune responses that participate in the activation of neutrophil granulocytes. The activation and degranulation of neutrophil granulocytes, as well as immune function mediated by neutrophil granulocytes, were significantly enriched, indicating that the immune microenvironment regulated CM and was important for tumor immunity. The neutrophil granulocytes of humans contain one type of a releasable membrane-bound organelle called a secretory granule and three main kinds of cytoplasmic granules; primary or azurophilic granules, secondary or specific granules, and third or gelatinase granules (27). The mature neutrophil granulocyte (polymorphonuclear neutrophils, neutrophil granulocyte, or neutrophil granulocyte-polymorphonuclears) occupies about 50–70% of the peripheral white blood cells of adults and is very rich in the tumor microenvironment (28). The neutrophil granulocyte is the first line of defense against the invasion of pathogens and also the earliest to immigrate to the site of inflammation (29). And the biological process with the highest concentration and components of cells in the GO terms showed that *SERPINA1* was strongly related to immune responses. Aggarwal *et al.* reported that massive cell proliferation and necroses of cells and tissues simulated the release of lysosomal protease, resulting in the compensatory increase of *SERPINA1* (30). The increased *SERPINA1* was attributed as anti-tumor by inducing infiltration of immune cells.

As far as we know, this study is the first to demonstrate that *SERPINA1* is strongly associated with CM prognosis. We found that a high expression of *SERPINA1* contributed to better survival rates in CM and was related to the infiltration of immune cells. Thus, *SERPINA1* might impact prognosis partially because of its relationship with the infiltration of immune cells.

However, this study had some limitations. Firstly, this study was retrospective, and all data were retrieved from an open database. Thus, our findings need to be replicated by external validation. Secondly, although we found *SERPINA1* was closely related to the infiltration of immune cells, further research into the mechanism involved will be worth pursuing.

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Footnote

Reporting Checklist: The authors have completed the TRIPOD reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-3873/rc

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-3873/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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