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Characteristics of tumor infiltrating lymphocytes in sinonasal mucosal melanoma and prognosis for patients

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A B S T R A C T

The significance of tumor infiltrating lymphocytes (TILs) in melanoma has been studied for a long time, but, to date, there has been insufficient research on TILs in sinonasal mucosal melanoma. The purpose of this study was to analyze the correlation between TILs and prognosis for Chinese patients with sinonasal mucosal melanoma, and to clarify the significance of TILs in prognosis. As a retrospective cohort study, 44 cases of malignant melanoma in head and neck mucosa were studied by immunohistochemical staining. The correlation between TIL classification (immune cell infiltration types), CD3, CD4, CD8, CD20, CD45, CD56, and CD68 positive cells, disease progression and prognosis for survival was analyzed. By pairing various factors, RNA sequencing and xCell analysis were performed in another 8 patients with different prognoses to further verify the expression of immune cell subsets in these patients. Immunohistochemistry and cell counting showed that the TIL classification and content of CD3, CD4, CD8, CD20, CD45, CD56, and CD68 positive cells were independent factors influencing progression-free survival, but there was no clear correlation with overall survival. RNA sequencing and xCell immunocyte analysis further confirmed the role of TILs in the prediction of disease progression. CD8+ T cells and natural killer T cells were highly expressed in patients with no disease progression, while Th2 T cells, macrophages and M2 macrophages were highly expressed in patients with disease progression. TILs can be used to predict the prognosis for patients with sinonasal mucosal melanoma. Different degrees and distributions of immune cell infiltration influence disease progression in patients with sinonasal mucosal melanoma. Patients with a diffuse distribution and a high density of infiltrating cells have a better prognosis. A high expression of CD8+ T cells

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and natural killer T cells, which have an immune killing effect, are beneficial in controlling progression of the disease.

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Introduction

The incidence of mucosal melanoma is low, but it is one of the common subtypes of melanoma in China accounting for about 23% of the total incidence of melanoma in the Chinese population.[1] As a result of the high degree of malignancy, the 5-year global overall survival rate is only about 20%.[2] Studies on tumor infiltrating lymphocytes (TILs) and prognosis in patients with melanoma have been carried out for a long time and most have confirmed that TILs are beneficial to the survival of patients.[3-5] In addition, TILs may be relevant markers to judge the prognosis and efficacy of immunotherapy.[6] However, most studies have not differentiated the subtypes of melanoma, and there are obvious differences in the biological characteristics of these subtypes. Exploring the characteristics of TILs in sinonasal mucosal melanoma will help to explain the poor efficacy of current stage treatments of this disease.

This study was directed at sinonasal mucosal melanoma, which is common in Chinese patients. The aim of this study was to analyze the correlation between TILs and disease disease progression and overall survival for Chinese patients with sinonasal mucosal melanoma using immunohistochemistry and transcriptome expression. The tumor microenvironment will be analyzed by IHC and cell counting to quantify TILs, and the effects of TILs on overall survival and disease progression will be analyzed Then, RNA sequencing and xCell will be used to verify the conclusions from immunohistochemistry. Thus, we hope to provide pathological and molecular evidence to further our understanding of the characteristics of the tumor microenvironment in sinonasal mucosal melanoma.

Data and methods

Patients samples inclusion

As a retrospective cohort study, 44 patients with sinonasal mucosal melanoma, who were treated in Beijing Tongren Hospital Affiliated to Capital Medical University from August 2010 to October 2018, were included in this study (Due to the low incidence rate and the long duration of patient sample collection, 44 patients were selected for the study.). Tumor tissue samples were collected and confirmed as mucosal melanoma by pathological diagnosis. In addition, 8 patients' tumor tissue were used to do the RNA sequencing. The 8 patients were matched by age, sex, primary location, pigment type, disease stage, operation mode and preoperative and postoperative adjuvant treatment but with different disease progression after surgery. The collection of tumor samples was approved by our hospital, and all patients gave their informed consent.

Immunohistochemistry

Tumor tissue samples were fixed with 4% paraformaldehyde, embedded in paraffin and sectioned with a thickness of 5 μm. Paraffin sections were dewaxed and hydrated with xylene and an ethanol gradient. After antigen repair with 0.01 mol/L citric acid buffer, the sections were

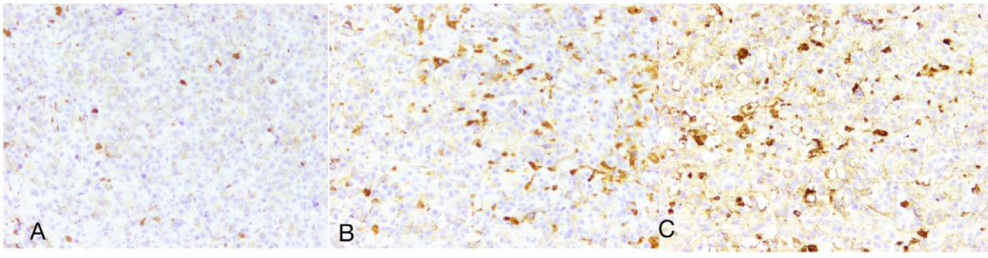

Fig. 1. Immunohistochemistry stain of CD45 under the light microscope. Type A: loss of immune cells; Type B: immune cells mainly concentrated in the stroma and perivascular tissue; Type C: diffuse immune cell infiltration. (Color version of figure is available online.)

tested with an immunohistochemistry kit (Abcam, Shanghai, China) using the following antibodies: anti-CD3 (Abcam, ab16669), anti-CD4 (Abcam, ab133616), anti-CD8 (Abcam, ab237709), anti-CD20 (Abcam, ab64088), anti-CD45 (Abcam, ab10558), anti-CD56 (Abcam, ab237708) and anti-CD68 (Abcam, ab955). The solutions were diluted at 1:50 and incubated overnight at 4°C. After phosphate-buffered saline (PBS) cleaning, biotin-labeled secondary antibody (concentration: 1:100) was added. After incubation at 37°C for 30 minutes, the tissue samples were stained with 3,3′-diaminobenzidine (DAB) substrate for 15 minutes, then washed with tap water, counterstained with hematoxylin, sealed with xylene transparent resin, then observed and photographed under a light microscope. According to the location of CD45, the types of immune cell infiltration were divided into the following 3 types: Type A: loss of immune cells; Type B: immune cells mainly concentrated in the stroma and perivascular tissue; Type C: diffuse immune cell infiltration (Fig 1). Five high power fields (HPF) were randomly selected from each section.

Cell counting method

Immunohistochemical staining was performed for CD3, CD4, CD8, CD20, CD45, CD56 and CD68. Under the microscope, the total number of cells per square millimeter (including melanoma cells) was estimated on representative tissue sections according to the staining of cell membrane markers. Using Cell Counter (University of Sheffield, Sheffield, UK), a square was drawn with an area of 0.04 mm^2, the cells were counted at 20× (SS), and multiplied by 25 to estimate the number of cells per square millimeter. The mean value was calculated from 5 samples.

RNA sequencing

The tumor tissue samples were cryopreserved at –80°C and RNA sequencing was performed after matching the 4 pairs patients having different disease progression. According to the manufacturer's protocol for the HiSeq 3000, sequencing system (Illumina, CA), the 100 bp paired-end protocol was used for sequencing to obtain the mRNAs of all samples. The short sequence obtained was read and compared with hg19 constructed by UCSC human genome using tophat2. The BAM file generated by the comparison was processed with htseq-count to calculate the count of each gene in all of the samples.

xCell

The tumor infiltrating immune cell model used was xCell (http://xcell.ucsf.edu/). The expression levels of 64 immune cell types in the tumor microenvironment were detected.[7] We submit-

ted the gene expression levels normalized to gene length (RPKM units) obtained from RNAseq to the xCell website and the enrichment score of each tissue was used to determine the *P*-value. $P < 0.05$ was considered to indicate statistically significant enrichment.

Statistical methods

Microsoft Excel 2017 (Microsoft Corp., Redmond, WA) was used to establish the database, and SPSS, version 24.0 statistical software (IBM Corp., Armonk, NY) was used to analyze the differences among the groups. The Chi-squared test was used to compare frequency data between groups. The Kaplan–Meier method and Cox regression analysis were used for survival and risk analysis. The log rank test was used for curve comparison between groups. The Cox regression model was used for risk factor analysis for significant variables obtained from univariate analysis. $P < 0.05$ was taken to indicate a statistically significant difference between groups.

Results

General data of the patients

Among the 44 patients, there were 22 men and 22 women. The onset age was 36-78 years, with a median age of 59.5 years. The patients were followed up to May 2021, with follow-up time ranging from 6-129 months. The primary sites of tumors were the nasal cavity (19), paranasal sinuses (3), nasal cavity and paranasal sinuses (17), nasopharynx (3), and other locations (2) as posterior end of nasal cavity / posterior edge of soft palate. According to the AJCC Cancer Staging Manual (eighth edn), there were 22 patients in stage III, 19 patients in stage IVA, and 3 patients in stage IVB. Among these patients 11 patients accepted postoperative chemotherapy, 8 patients accepted postoperative radiotherapy, 6 patients accepted postoperative concurrent chemoradiotherapy and 6 patients accepted postoperative immunotherapy.There were 27 patients with disease progression (postoperative lymph node metastasis, local recurrence, distant metastasis) and 17 patients with no disease progression (Supplement Table 1).

In addition, all 8 patients (4 men and 4 women) were initially treated by surgery in our hospital between May 2020 and September 2020. The age at onset of disease ranged from 47-76 years, with a median age of 62 years. These 8 patients were followed up until May 2021 giving a follow-up period ranging from 8 to 11 months. The primary lesions were located in the nasal cavity and paranasal sinuses. There were 2 patients in stage III, 2 patients in stage IVA and 4 patients in stage IVB. At the end of follow-up, 4 patients had tumor progression (postoperative lymph node metastasis, local recurrence, distant metastasis), 4 patients had no disease progression, and 3 patients had died.

Immunotyping and survival

According to the previously outlined classification of immune cell infiltration (Type A: loss of immune cells; Type B: immune cells mainly concentrated in the stroma and perivascular tissue; Type C: diffuse immune cell infiltration, according to the location of CD45), the percentages of type A, B and C immune cell infiltration were 31.82%, 50% and 18.18%, respectively (Fig 2A). It can be seen that the main types of sinonasal mucosal melanoma are type A and type B (Fig 1A), and the overall immune cell infiltration lower than other solid tumor. The correlation of general clinical characteristics and disease progression (lymph node metastasis, local recurrence, distant metastasis) with immune cell infiltration types in patients were analyzed statistically, and the results for univariate analysis are shown in Table 1. There was no correlation between the types of immune cell infiltration and the general clinical characteristics of the disease, but there were significant differences in disease progression among the different immune cell infiltration types.

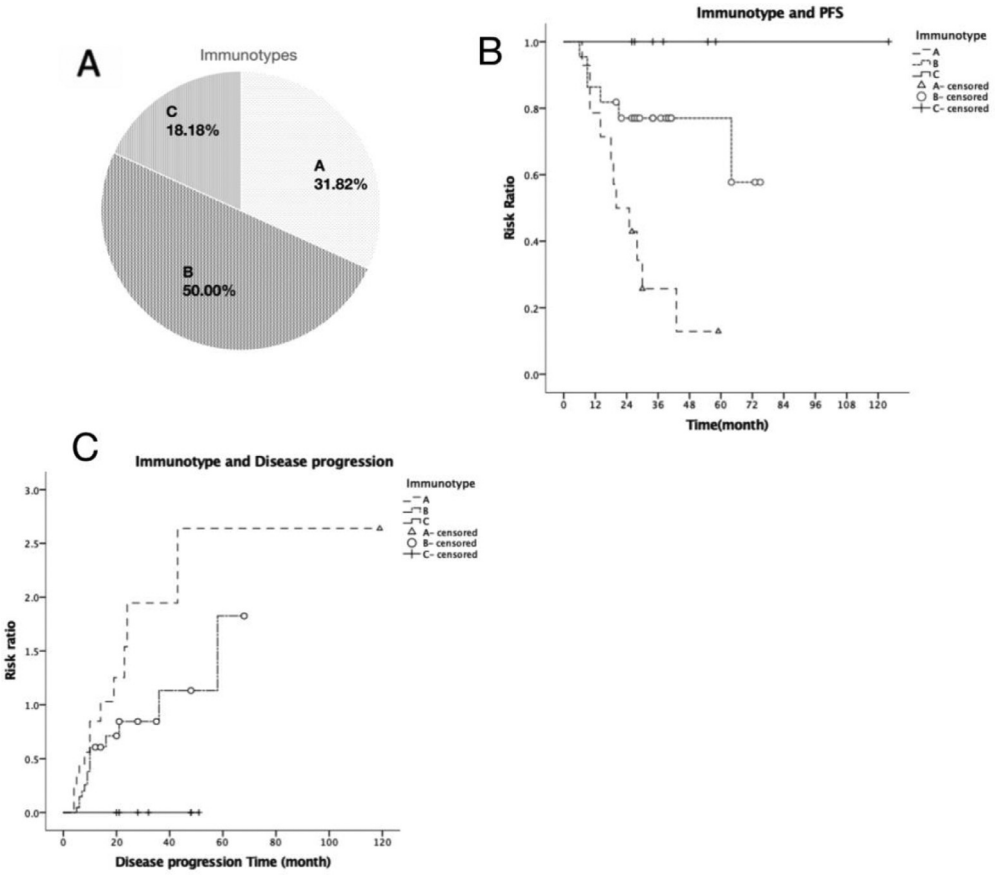

Fig. 2. (A) Proportions of the 3 types of immune cell infiltration; (B) Difference in PFS among patients with different types of immune cell infiltration (median PFS time: A: 19.5 mo; B: 21 mo; C: 24 mo); (C) Correlation curves between immune cell infiltration types and disease progression.

Table 1
Univariate analysis of general factors and immune cell infiltration types.

Factor		Immunotype			*P*
		A	B	C	
Age	<60	6	11	6	0.333
	≥60	8	11	2	
Sex	Male	6	11	5	0.675
	Female	8	11	3	
Stage	III	4	14	4	0.11
	IVA	10	6	3	
	IVB	0	2	1	
Pigment	with	10	19	7	0.474
	without	4	3	1	
Site	Nose cavity	5	11	3	0.445
	Sinuses	2	1	0	
	Nasal cavity and sinuses	6	6	5	
	Nasopharynx	0	3	0	
	Others	1	1	0	
Disease progression	Yes	13	14	0	0.02
	No	1	8	8	

Table 2
Multivariate analysis of progression-free survival (PFS) and overall survival (OS) with immune cell infiltration type.

Factor	PFS		OS	
	Exp (B) (95%CI)	*P*	Exp (B) (95%CI)	*P*
Disease progression	——	——	12.844 (2.401-68.697)	0.003
Postoperative chemotherapy	0.409 (0.173-0.972)	0.043	0.203 (0.044-0.930)	0.040
Stage	0.198 (0.040-0.995)	0.049	0.063 (0.007-0.607)	0.048
Immunotype	0.419 (0.211-0.830)	0.013	——	0.759

Table 3
Numbers of immune cells by immunotype.

	Immunotype A (Mean)	Immunotype B (Mean)	Immunotype C (Mean)	*P* (A vs B)	*P* (B vs C)
CD45	15.30	398.20	706.33	0.00	0.00
CD3	12.21	290.00	550.00	0.00	0.00
CD8	7.20	123.33	330.00	0.00	0.00
CD4	6.15	175.00	245.00	0.00	0.00
CD20	4.60	98.20	210.55	0.00	0.00
CD68	16.87	39.45	40.175	0.00	0.87
CD56	0.52	2.31	5.27	0.00	0.00

Univariate analysis of progression-free survival (PFS) and overall survival (OS) was conducted on all possible related factors: age, sex, site, pigment, stage, postoperative adjuvant therapy (postoperative chemotherapy, postoperative radiotherapy, postoperative concurrent chemoradiotherapy, postoperative immunotherapy), disease progression (lymph node metastasis, local recurrence, distant metastasis) and immune cell infiltration type. Univariate analysis showed that stage and disease progression may be risk factors for worsen PFS and OS. Postoperative chemotherapy, immune cell infiltration types were associated with better PFS and OS. (a table with univariate analysis data is not included due to space limitations). The possible risk factors identified from univariate analysis were included in the multivariate analysis (Table 2). The results showed that postoperative chemotherapy, stage and immune cell infiltration types were all independent factors influencing PFS. The median PFS times for patients with immunotype A, B and C were 19.5 months 21 months and 24 months, respectively (Fig 2B). Disease progression, postoperative chemotherapy and stage were the independent factors affecting OS, while immune cell infiltration types were not independent factors affecting OS. The correlation curves for the risk ratio of immune cell infiltration type with PFS and immune cell infiltration type with disease progression are shown in Figure 2B-C, respectively.

This shows that the different distributions of immune cell infiltration influence disease progression in patients with sinonasal mucosal melanoma.

Content of immune cell subtypes and survival

Combined analysis of cell counting and the mean value (Table 3) with immune cell infiltration types. It can be seen that the number of cells for each cell marker from type A, B, and C increases in turn. Also, there is a significant statistical difference of cell numbers between different subtypes. The results showed that the distribution of cell content of each immune cell subtype was consistent with that of immune cell infiltration types.

The relationship between the number of CD3, CD8, CD4, CD20, CD45, CD56, and CD68 positive cells and PFS and OS was analyzed. Univariate analysis was conducted on possible related factors: age, sex, site, pigment, stage, postoperative adjuvant therapy (postoperative radiotherapy, postoperative chemotherapy, postoperative concurrent chemoradiotherapy, postoperative immunotherapy), disease progression (lymph node metastasis, local recurrence, distant metastasis) and content of each immune cell subtypes. Univariate analysis showed that stage and

Table 4
Multivariate analysis of disease progression and overall survival with immune cells.

Factor	Disease progression (Multivariate)		Overall Survival (Multivariate)	
	Exp (B) (95%CI)	*P*	Exp (B) (95%CI)	*P*
Disease progression	——	——	12.365 (2.290-66.779)	0.003
Postoperative chemotherapy	0.404 (0.170-0.960)	0.040	0.204 (0.045-0.924)	0.039
Stage	0.195 (0.039-0.998)	0.048	0.066 (0.007-0.615)	0.048
CD45	0.998 (0.996-0.999)	0.011	0.998 (0.994-1.002)	0.358
CD3	0.997 (0.994-0.999)	0.008	0.997 (0.991-1.002)	0.218
CD8	0.992 (0.987-0.998)	0.005	0.992 (0.981-1.004)	0.204
CD4	0.994 (0.989-0.999)	0.017	0.992 (0.981-1.003)	0.159
CD68	0.990(0.983-0.997)	0.008	0.990 (0.975-1.005)	0.193
CD56	2.569(0.324-3.131)	0.018	0.956(0.802-2.158)	0.351
CD20	1.021 (0.989-1.054)	0.198	16.950 (3.195-89.929)	0.617

Table 5
TIL subtype xCell score expression between the disease progression and disease progression-free.

	Disease progression (Average)	Disease progression-free (Average)	*P*
CD4+T cell	2.445	0.587	0.159
CD4+Tcm	0.937	0.490	0.528
CD4+Tem	3.421	4.620	0.607
Th1 T cell	0.969	0.630	0.103
Th2 T cell	1.636	0.297	0.033
CD8+T cell	0.240	4.128	0.007
CD8+Tcm	2.264	0.409	0.122
CD8+Tem	3.435	5.480	0.076
NKT cell	0.040	0.551	0.003
Macrophages	0.458	0.026	0.001
M1 macrophages	0.059	0.032	0.383
M2 macrophages	0.508	0.013	0.007
Plasma cells	0.037	0.013	0.120
B cells	0.026	0.017	0.701

disease progression may be risk factors for worsen PFS and OS. And postoperative chemotherapy, the number of CD45, CD3, CD8, CD4, CD20, CD56, CD68 positive cells were associated with better PFS and OS (a table with univariate analysis data is not included due to space limitations). The possible risk factors identified from univariate analysis were included in the multivariate analysis (Table 4). The results showed that postoperative chemotherapy, stage, and the number of CD45, CD3, CD8, CD4, CD56 and CD68 positive cells were all independent factors influencing disease progression. However, the numbers of only some of these positive cells were independent factors for OS (Table 4).

xCell immunoassay

We screened the 4 pairs of patients with different prognoses (with or without disease progression) for RNA sequences. We further determined the expression score of immune cell subtypes using the xCell method, and from this analysis the immune cells were subdivided into the following immune cell subtypes: CD4+T cell, CD4+ Tcm, CD4+ Tem, Th1 T cells, Th2 T cells, CD8+ T cell, CD8+ Tcm, CD8+ Tem, natural killer T (NKT) cells, macrophages, macrophage M1 cells, macrophage M2 cells, plasma cells and B cells. The specific differences in cell expression score in patients with or without disease progression are shown in Figure 3. CD8+T cells and NKT cells were highly expressed in patients with no disease progression, while Th2 T cells, macrophages and M2 macrophages were highly expressed in patients with disease progression after surgery. Also, the differences were statistically significant (Table 5). In general, CD8+ T

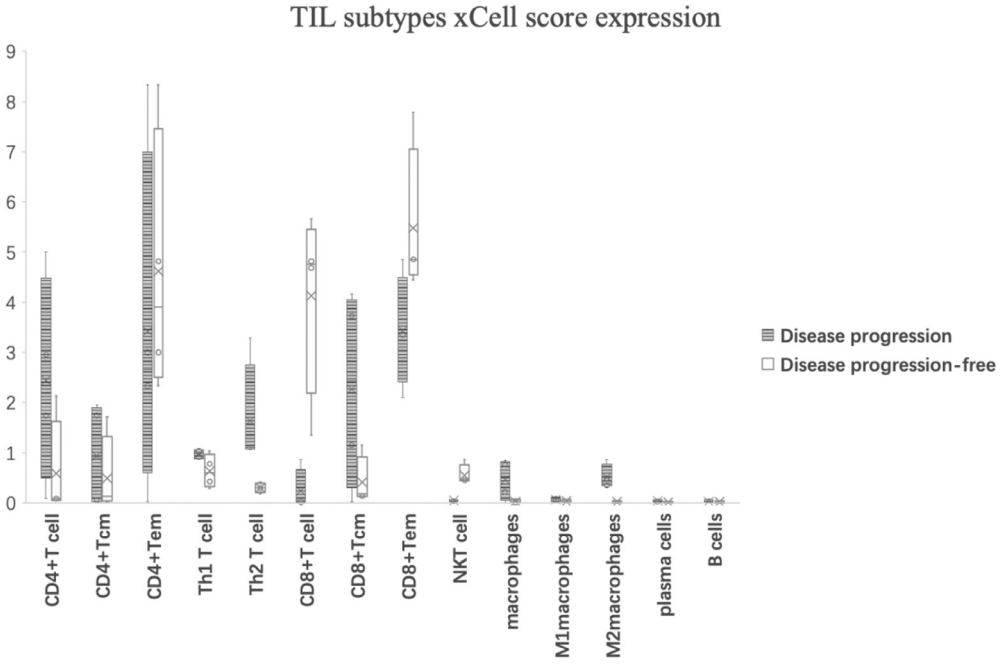

Fig. 3. TIL subtypes xCell score expression between the disease progression and disease progression-free group.

cells and NKT cells as immune killer cells with a positive immune effect are relatively highly expressed in patients without disease progression, which also confirms the beneficial effect of active autoimmune activity on disease progression. The high expression of Th2 T cells and M2 macrophages in patients with postoperative disease progression is also consistent with the immunosuppressive mechanism of these cells.

Discussion

TILs are a group of heterogeneous and anti-tumor lymphocyte populations in tumor tissue.[8] In the tumor microenvironment, TILs are affected by different cell activation mechanisms and cytokines, which can produce different immune responses[9] and affect the occurrence and development of tumors. Research into the prognostic role of TIL in solid tumors has been carried out for many years, and there are many ways to evaluate the location and degree of cell infiltration. The classification of TILs in melanoma was proposed by scholars as early as the 1970s.[10] The methods used to evaluate TILs include the non-quantitative grading standards proposed by Clark.[10] and Azimi[11], and the following approximate quantitative grading standards: the Saldanha system.[12] or TCGA/Park system.[13] The international immuno-oncology biomarkers working group proposed a classification system for TILs in all solid tumors (including melanoma).[14] That system defined the location of the tumor boundary, and the methods used to evaluate TILs in the stroma and tumor. However, because of the uniqueness of the anatomic structure of sinonasal mucosal melanoma, it is difficult to define the boundary of mucosal lesions, so it is difficult to apply the existing TIL classification methods to solid tumors. Therefore, the prognostic value of the TIL classification in patients with sinonasal mucosal melanoma is still unclear, and very few studies have examined this subtype. The method of evaluation used in this study is similar to the conventional immunohistochemical interpretation methods. Depending on the location of CD45 markers, there were 3 different types of immune cell infiltration: Type A: loss of immune

cells; Type B: immune cells mainly concentrated in the stroma and perivascular tissue; Type C: diffuse immune cell infiltration. This evaluation method has also been verified in earlier studies on melanoma.[15]

For the prognostic significance of TIL in melanoma, most studies have confirmed that the degree of invasion is positively correlated with the prognosis for patients. A study of 4133 patients with primary cutaneous melanoma showed that the survival rate increased with increasing TIL levels ($P = 0.0001$).[16] A meta-analysis of the role of TIL in the prognosis for melanoma showed that TILs had a positive effect on recurrence-free survival.[HR: 0.72 (0.58-0.90)].[17] These results indicate that the TIL level in the immune microenvironment affects the survival and disease progression in patients with melanoma. However, the biological characteristics of mucosal melanoma are different from skin melanoma, for example, low tumor load, so there may also be differences in the local immune environment of the tumor. Some scholars used immunohistochemistry to evaluate the difference of CD4+ TILs, CD8+ TILs and FoxP3+ regulatory T cells (Tregs) in acral and mucosal melanoma with skin melanoma.[18] The results showed that the total number of TILs and CD8+ TILs in acral and mucosal melanoma were significantly lower than those in cutaneous melanoma. The number and proportion of Tregs in CD4+ T cells in mucosal melanoma were significantly higher than those in skin and acral melanoma. These results indicate that the overall level of TILs in mucosal melanoma is lower than in other subtypes of melanoma, and the levels of immunosuppressive cells are increased in mucosal melanoma, and this may affect the prognosis and treatment of patients. In addition to the lower levels of TILs in mucosal melanoma, we also found that type A (loss of immune cells) and type B (immune cells mainly concentrated in the stroma and around the blood vessels) are the main types of sinonasal mucosal melanoma, and, in our study, the distribution of these immune cells has a clear correlation with the progression of postoperative disease. These results indicate that, in addition to the absence of immune cells, the migration of immune cells to tumors is also hindered. In addition, the distribution of immune cells has a clear correlation with disease progression after surgery. During the process of immune cells penetrating blood vessels and migrating to the tumor, any therapeutic intervention may improve the prognosis for patients with sinonasal mucosal melanoma.

In the studies on TIL cell infiltration, a paper reporting 35 cases of mucosal and acral melanoma identified that the density of immune cells, especially CD8+ T cells, was significantly correlated with the survival rate of patients.[19] A study involving 82 patients with oral mucosal melanoma also showed that TIL was an independent risk factor for distant metastasis, and patients with no TIL infiltration had a higher risk of distant metastasis.[20] Our study on cell counting also confirmed that the greater the number of CD45, CD8, CD3 and other lymphocytes, the better the control of disease progression. The distribution of cell content was consistent with TIL classification types. CD3, CD45, CD4, CD8, CD20, CD56 and CD68 were used as markers of T cells, plasma cells, NKT cells and macrophages for immunohistochemical detection. In our study, CD3, CD45, CD4, CD8, CD56 and CD68 were found to have an effect on progression of the disease. However, because these markers on the cell surface are not the only ones present, this method of analysis based on immunohistochemistry is biased.

In addition to traditional immunohistochemical research with the application of artificial intelligence algorithms in recent years, the method of estimating TIL components using RNA expression is gradually maturing. RNA expression data such as CIBERSORT TIMER, etc. is used to estimate tumor cell composition.[21,22] In our study, the xCell algorithm.[23] was used to evaluate the immune components of transcriptome data from paired patients with different prognoses. Based on the transcriptome data, the immune cell subtypes were refined to explore the distribution characteristics of immune cells in patients with different prognoses. We identified the marker genes of each cell and used xCell to calculate the score of immune cells. We found that CD8+ T cells and NKT cells were highly expressed in patients with no disease progression after surgery, and Th2 cells, macrophages and M2 macrophages were highly expressed in patients with disease progression after surgery. In our study, it can be seen that the expression of different immunohistochemical markers is easily obstructed by different cell subsets. We cannot simply rely on a wide range of markers to judge the role of CD4 and CD8 cells in the tumor mi-

croenvironment, but need more detailed analysis of cell subtypes. But in general, CD8+ T cells, NKT and other immune killer cells with a positive immune effect are relatively highly expressed in patients without disease progression.

CD8+ T cells are generally considered to be an important type of immune cell that control tumor growth and have strong cytotoxicity.[23] NKT cells can also kill different types of tumor cell[24], and both of these cell types can exert anti-tumor effects through downstream MHC I molecules. A high density of tumor infiltrating NKT cells has been proved to be related to good prognosis for a variety of solid tumors.[25] The distribution characteristics of CD8+T cells and NKT cells obtained from xCell analysis not only agree with the conclusions of previous immunohistochemical studies, but also with the roles of the 2 kinds of cells in tumor immunity in other studies. For CD4+ T cells and macrophages, there are many subtypes of CD4+ T cells and macrophages, and the characteristics of different subtypes are also complex. These different cell types perform specific immune regulatory functions to enhance or inhibit immune response.[26]

The main helper cell subtypes are Th1 and Th2 cells. Th1 cells are considered to be the most important helper cell type in tumor immunity. They participate in the killing of tumor cells, secrete cytokines, activate death receptors on the surface of tumor cells and induce the diffusion of epitopes.[27] The persistence of Th2 cells in vivo is related to long-term immunity. Macrophages also show 2 immune functions. Mantovani and colleagues.[28] considered that macrophages have a series of continuous functional states, and M1 and M2 macrophages are the 2 extremes of this continuous state. M1 macrophages play an important role in immune surveillance by secreting pro-inflammatory cytokines and chemokines and presenting antigens; M2 macrophages only have weak antigen presenting ability, and down-regulate immune response by secreting inhibitory cytokines such as IL-10 or TGF-β.[29] Immunohistochemistry only identified the difference in expression of CD4 and CD68 positive cells in patients with different prognoses, while RNA sequencing further explored the role of specific subtypes. It was found that the high expression of Th2, macrophages and M2 macrophages was more common in patients with disease progression after surgery, which is also consistent with the immune function of CD4+ T cells and macrophages mentioned above. Therefore, immunohistochemical analysis will be affected by different cell subtypes expressing the same cell surface antigen, and it is necessary to discuss the role of subtypes in tumor immunity. In this respect, gene level analysis seems to be more conducive to the comprehensive evaluation of TILs.

With the application of immunotherapy, researchers have linked TILs with the efficacy of immunotherapy, and identified tumors as inflammatory/hot or non-inflammatory/cold. High CD8+ T cell density and PD-L1 expression in the tumor microenvironment have been widely used as biomarkers to evaluate the response of immunotherapy drugs.[30] In addition, adoptive cell therapy also plays a research and therapeutic role through TIL-mediated recognition and elimination of solid tumors[31], and the development of these therapies in sinonasal mucosal melanoma requires further basic and in-depth research.

Generally, with our improved understanding of the role of TIL in tumors and further study of the tumor immune microenvironment, researchers will not have to rely on only the evaluation of TIL immune molecular markers to predict prognosis. In the future, comprehensive analysis of TIL location, density, and other interstitial components in the tumor microenvironment and immunosuppressive components will be more conducive to the evaluation of a patient's immune status. It is necessary for further screening and research of new therapeutic targets. We will also study the differences in type, distribution and content of immune cells in order to determine the role of molecular mediated genes in these processes and provide better intervention treatment. This will help to improve the prognosis for patients with sinonasal mucosal melanoma. Our study has several limitations. Because the incidence of sinonasal mucosal melanoma is very low, the number of patients in this study is limited. As a result, the sample size for RNA sequencing after matching is small, which may bias the conclusions. Our research center is committed to research into sinonasal mucosal melanoma. As the largest diagnostic and treatment center for sinonasal mucosal melanoma in China, we will continue to collect cases for further research.

Conclusion

Tumor infiltrating lymphocytes can be used to predict the prognosis for patients with sinonasal mucosal melanoma. Different degrees and distributions of immune cell infiltration have a certain influence on disease progression in patients. Patients with a diffuse distribution and a high density of infiltrating cells have a better control of disease progression, and a high expression of CD8+ T cells and NKT cells, which have an immune killing effect, are beneficial in controlling progression of the disease.

Conflict of interest

The authors declare that they have no known competing financial interests or individual relationships to disclose.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.currproblcancer.2022.100878.

References

1. Chang AE, Karnell LH, Menck HR. The national cancer data base report on cutaneous and noncutaneous melanoma: a summary of 84,836 cases from the past decade. The American college of surgeons commission on cancer and the American cancer society. *Cancer*. 1998;83:1664–1678.
2. Mihajlovic M, Vlajkovic S, Jovanovic P, et al. Primary mucosal melanomas: a comprehensive review. *Int J Clin Exp Pathol*. 2012;5:739–753.
3. Chen K, Cheng G, Zhang F, et al. PD-L1 expression and T cells infiltration in patients with uncommon EGFR-mutant non-small cell lung cancer and the response to immunotherapy. *Lung Cancer*. 2020;142:98–105.
4. Fridman WH, Pagès F, Sautès-Fridman C, et al. The immune contexture in human tumours: impact on clinical outcome. *Nat Rev Cancer*. 2012;12:298–306.
5. Kashiwagi S, Asano Y, Goto W, Asano Y, et al. Using TILs to Predict Therapeutic Effect of Chemotherapy (Pertuzumab, Trastuzumab, Docetaxel) on HER2-positive Breast Cancer. Anticancer Res. *Anticancer Res*. 2017 Oct;37(10):5623–5630. doi:10.21873/anticanres.11997.
6. Outh-Gauer S, Morini A, Tartour E, et al. The microenvironment of head and neck cancers: papillomavirus involvement and potential impact of immunomodulatory treatments. *Head Neck Pathol*. 2020;14:330–340.
7. Aran D, Hu Z, Butte AJ. xCell: digitally portraying the tissue cellular heterogeneity landscape. *Genome Biol*. 2017;18:220.
8. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? *Lancet*. 2001;357:539–545.
9. Duraiswamy J, Kaluza KM, Freeman GJ, et al. Dual blockade of PD-1 and CTLA-4 combined with tumor vaccine effectively restores T-cell rejection function in tumors. *Cancer Res*. 2013;73:3591–3603.
10. Clark WH, From L, Bernardino EA, et al. The histogenesis and biologic behavior of primary human malignant melanomas of the skin. *Cancer Res*. 1969;29:705–727.
11. Azimi F, Scolyer RA, Rumcheva P, et al. Tumor-infiltrating lymphocyte grade is an independent predictor of sentinel lymph node status and survival in patients with cutaneous melanoma. *J Clin Oncol*. 2012;30:2678–2683.
12. Saldanha G, Flatman K, Teo KW, et al. A novel numerical scoring system for melanoma tumor-infiltrating lymphocytes has better prognostic value than standard scoring. *Am J Surg Pathol*. 2017;41:906–914.
13. Park CK, Kim SK. Clinicopathological significance of intratumoral and peritumoral lymphocytes and lymphocyte score based on the histologic subtypes of cutaneous melanoma. *Oncotarget*. 2017;8:14759–14769.
14. Hendry S, Salgado R, Gevaert T, et al. Assessing tumor-infiltrating lymphocytes in solid tumors: a practical review for pathologists and proposal for a standardized method from the international immuno-oncology biomarkers working group: part 2: TILs in melanoma, gastrointestinal tract carcinomas, non-small cell lung carcinoma and mesothelioma, endometrial and ovarian carcinomas, squamous cell carcinoma of the head and neck, genitourinary carcinomas, and primary brain tumors. *Adv Anat Pathol*. 2017;24:311–335.
15. Erdag G, Schaefer JT, Smolkin ME, et al. Immunotype and immunohistologic characteristics of tumor-infiltrating immune cells are associated with clinical outcome in metastatic melanoma. *Cancer Res*. 2012;72:1070–1080.
16. Fortes C, Mastroeni S, Mannooranparampil TJ, et al. Tumor-infiltrating lymphocytes predict cutaneous melanoma survival. *Melanoma Res*. 2015;25:306–311.
17. Fu Q, Chen N, Ge C, et al. Prognostic value of tumor-infiltrating lymphocytes in melanoma: a systematic review and meta-analysis. *Oncoimmunology*. 2019;8:1593806.
18. Nakamura Y, Zhenjie Z, Oya K, et al. Poor lymphocyte infiltration to primary tumors in acral lentiginous melanoma and mucosal melanoma compared to cutaneous melanoma. *Front Oncol*. 2020;10:524700.

19. Edwards J, Ferguson PM, Lo SN, et al. Tumor mutation burden and structural chromosomal aberrations are not associated with T-cell density or patient survival in acral, mucosal, and cutaneous melanomas. *Cancer Immunol Res*. 2020;8:1346–1353.
20. Song H, Wu Y, Ren G, et al. Prognostic factors of oral mucosal melanoma: histopathological analysis in a retrospective cohort of 82 cases. *Histopathology*. 2015;67:548–556.
21. Li B, Severson E, Pignon J-C, et al. Comprehensive analyses of tumor immunity: implications for cancer immunotherapy. *Genome Biol*. 2016;17:14.
22. Newman AM, Liu CL, Green MR, et al. Robust enumeration of cell subsets from tissue expression profiles. *Nat Methods*. 2015;12:453–457.
23. Iwahori K. Cytotoxic CD8+ lymphocytes in the tumor microenvironment. *Adv Exp Med Biol*. 2020;1224:53–62.
24. Vitale M, Cantoni C, Pietra G, et al. Effect of tumor cells and tumor microenvironment on NK-cell function. *Eur J Immunol*. 2014;44:1582–1592.
25. Denkert C, von Minckwitz G, Darb-Esfahani S, et al. Tumour-infiltrating lymphocytes and prognosis in different subtypes of breast cancer: a pooled analysis of 3771 patients treated with neoadjuvant therapy. *Lancet Oncol*. 2018;19:40–50.
26. Kennedy R, Celis E. Multiple roles for CD4+ T cells in anti-tumor immune responses. *Immunol Rev*. 2008;222:129–144.
27. Knutson KL, Disis ML. Tumor antigen-specific T helper cells in cancer immunity and immunotherapy. *Cancer Immunol Immunother*. 2005;54:721–728.
28. Martinez FO, Sica A, Mantovani A, et al. Macrophage activation and polarization. *Front Biosci*. 2008;13:453–461.
29. Murray PJ, Allen JE, Biswas SK, et al. Macrophage activation and polarization: nomenclature and experimental guidelines. *Immunity*. 2014;41:14–20.
30. Keenan TE, Burke KP, et al. Genomic correlates of response to immune checkpoint blockade. *Nat Med*. 2019;25:389–402.
31. Feldman SA, Assadipour Y, Kriley I, et al. Adoptive cell therapy–tumor-infiltrating lymphocytes, T-cell receptors, and chimeric antigen receptors. *Semin Oncol*. 2015;42:626–639.