Immunosuppressive phenotype of esophagus tumors stroma

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Research article

Keywords: esophageal squamous cell carcinoma, macrophage, T-cell, stroma

Posted Date: June 1st, 2020

DOI: https://doi.org/10.21203/rs.3.rs-30657/v1

Abstract

Background

Tumor associated macrophages and tumor infiltrating lymphocytes contribute significantly to the development of immunosuppressive properties of tumor. In this study we performed immunohistochemical analysis of immune cells of esophageal tumors stroma.

Methods

Paraffin-embedded tissue specimens from 48 esophageal squamous cell carcinoma patients were retrospectively collected for immunohistochemical analysis of stromal cells. For staining of macrophages, CD68, CD163, CD206, PU.1 and iNOS were used. For T-cells detection CD8, CD3, FOXP3 were used. As well we performed staining for PD-L1 that can be expressed on tumor associated macrophagesand tumor cells. Clinicopathological and survival data were collected and analyzed using the χ^2 and Fisher exact tests, Kaplan–Meier curves, and the log-rank test. The correlation analysis was performed with Spearman correlation coefficient.

Results

The level of CD206 expression was associated with histological grade (p = 0.034), FOXP3 expression was associated with sex and age (p = 0.041, p = 0.003 respectively) and iNOS expression was associated with the disease stage (p = 0.044). In addition, FOXP3 and CD163 appeared to be markers of good prognosis (HR = 0.5407, p = 0.0462; HR = 0.4447, p = 0.0456 respectively). Significant association between PU.1 + and CD68 + macrophages (r = 0.752; P = 0.000) and between PU.1 + and CD163 + macrophages (r = 0.585; P = 0.000) was established, positive association between PU.1 and CD206 expression was also observed (r = 0.424; P = 0.001).

Conclusions

Large amounts of CD163 + macrophages and FOXP3+ T-cells appear to be markers of good prognosis of esophageal squamous cell carcinoma. The number of PU.1 + macrophages strongly correlate with the number of CD68 + macrophages therefore usage of PU.1 as a potential macrophage marker can be recommended esophageal tumors.

Background

Esophageal cancer is the sixth frequent cause of death among malignant tumors. Due to late stage diagnosis about 70% of patients die within 1 year after diagnosis. There are two main subtypes of esophageal cancer described: esophageal squamous cell carcinoma (ESCC) comprising 90% esophageal cancer cases and esophageal adenocarcinoma (EAC).

Available data indicate that in order to understand the pathogenesis of esophageal cancer, it is necessary not only to understand the molecular repertoire of the tumor cells, but also the properties of the cells of the tumor microenvironment, which contains various cells of the immune system that support the development of the tumor at all its stages. The escape of the tumor from immunological control is crucial for the survival, progression and metastasis of the tumor. Tumor cells can suppress the antitumor immune response through the production of various soluble factors, which, in turn, attract and direct the differentiation and activation of stromal cells in the direction necessary for the tumor. In this work, we examined cell populations associated with the tumor immune escape, namely macrophages and T cells.

TAMs show a number of pro-tumorigenic features. It is widely accepted that macrophages may display a broad spectrum of phenotypes where widely accepted type 1 (M1) and type 2 (M2) macrophages represent its extremes. M1 stimulate inflammation, produce pro-inflammatory cytokines, and show anti-tumor cytotoxic activity, M2 produce anti-inflammatory cytokines, extracellular matrix components and remodeling enzymes, show high phagocytic and low cytotoxic activities(1–3). TAMs support tumor progression by producing proangiogenic and growth factors. They also thought to inhibit T-cell effector functions by releasing immunosuppressive cytokines(3, 4). In most of studied cancers the presence of increased number of TAMs appears to be a marker of poor prognosis. This is also the case for esophageal cancer(5).

Tumor-infiltrating lymphocytes (TILs) represent another important part of tumor stroma cells. They are found in different tumors and their population is mainly comprised of CD3 + and CD8 + T cells. CD3 + T cells have antitumor activity(6). As TAMs, CD3 + TILs show both anti-tumor and tumor supporting activities. In contrast, CD8 + T lymphocytes have cytotoxic activity against cancer cells, and these T cells could play an important role in antitumor immunity. Regulatory T cells (Tregs) also show immunosuppressive activity in cancer. In a healthy organism, Tregs control activation and expansion B and T cells, as well as NK cell cytotoxicity; however, in cancer they inhibit anti-tumor immune responses(7). Interestingly, Tregs may act differently at different stages of tumor development. At the initial stages Tregs suppress inflammation that may lead to carcinogenesis, but later diminishing anti-tumor immunity via the secretion of immunosuppressive cytokines and inhibition of cytotoxic cell function(8).

Recent advances in cancer immunological therapeutics have revealed the importance of programmed death-1 (PD-1) – activated signaling. The combination of PD-1 and its ligand PD-L1 is the key immune checkpoint for inhibition of T-cell activation. Recently developed PD-L1 inhibitor antibodies are now used for treatment of various cancers including esophageal cancer. However, in contrast to many other tumors the association of PD-L1 expression with the clinicopathological relationship in ESCC remains controversial. Some studies demonstrated that PD-L1 expression correlates with poor prognosis(9), while others suggested that PD-L1 could be a favorable prognostic indicator in ESCC(10).

In this study we have examined the prognostic impact of different components of tumor stroma basing on immunohistochemical analysis of macrophage and T-cell markers in a group of 48 curatively resected esophageal squamous cancers. We established that out of all macrophage markers studied only CD206 correlates with the clinicopathological features of the tumor. Analysis of survival revealed that the number of CD163 + TAMs and FOXP3 + TILs correlates with prolonged survival of the patients. We also tested PU.1 as a potential general marker for macrophages and demonstrated its high correlation with CD68, what confirms our hypothesis of the possible use of nuclear PU.1 staining for labelling TAMs.

Materials And Methods

Study population

Human ESCC tissues in form of formalin-fixed paraffin-embedded (FFPE) sections were obtained from the Clinical Oncology department of N. N. Blokhin Russian Cancer Research Centre (Moscow, Russia). In total 48 samples were collected from the patients that underwent treatment between 2005 and 2012. Detailed description of the study population is presented in the Supplementary table 1. The survival status of the patients was followed up by post until February 2020. The median follow-up for patients still alive was 40 months (range, 2–152 months). Overall survival (OS) was defined as the interval between surgery and death or between surgery and the last follow-up for surviving patients. Among the 37 patients, 23 (62.0%) died, and 14 (38.0%) were alive during the follow-up period.

The Institutional Review Board of N.N. Blokhin Russian Cancer Research Center approved the project (approval date 09/2018) and all patients, who were involved in the study, gave written informed consents that their samples could be used for research purposes. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki. Data were analyzed anonymously. All potential participants who declined to participate or otherwise did not participate were eligible for treatment (if applicable) and were not disadvantaged in any other way by not participating in the study.

Immunohistochemical analysis

Standard immunohistochemical procedure was used for staining FFPE sections with antibodies against stromal cells markers. We used following antibodies: mouse anti-CD163 (Clone 10D6; BIOCARE, USA, 1:100 dilution), rabbit anti-CD206 (HPA004114; Sigma, USA, 1:2000 dilution), rabbit anti-iNOS (SAB5500152; Sigma, USA, 1:150 dilution), rabbit anti-FOXP3 (Clone D2W8E; Cell Signaling Technology, USA, 1:200 dilution), rabbit PU.1 (Clone 9G7; Cell Signaling Technology, USA, 1:200 dilution), rabbit anti-PD-L1 (E1L3N; Cell Signaling Technology, USA, 1:200 dilution), rabbit anti-CD68 (clone GR021, 61–0184 Genemed, USA, 1:100 dilution), mouse anti-CD8 (clone CD8/144B, 61–0124 Genemed, USA, 1:100 dilution) and rabbit anti-CD3 (61 – 0011 Genemed, USA, 1:100 dilution). We used UltraVision Quanto Detection System HRP DAB (Thermo Fisher Scientific, USA).

Evaluation of macrophages and T cells

Macrophages and T-cells were counted in tumor islets and in stroma. The numbers of CD68, CD163, CD206, CD3 and CD8 positive cells in immunohistochemical staining were counted in 5 independent high-power microscopic fields (400×) of tumor tissue. The mean percentages of stained cells were scored as 0 (negative), 1 (≤ 10%), 2 (11–50%), 3 (> 50%). Further the samples with low number of positive cells (scores 0–1) were grouped in a low expression group, and samples with the scores 2–3 in a high expression group. FOXP3 и PU.1 are transcription factors, therefore only nuclear expression was counted. FOXP3 positive cells were counted at a magnification of 400×. FOXP3 expression was assessed according to the number of positive cells in 5 randomly chosen fields. The median number of FOXP3 positive cells was used as a cutoff to divide samples into FOXP3 low and FOXP3 high groups. The analysis of PU.1 expression was done the same was as for the other macrophage markers studied here. For iNOS the sample was considered to have low expression if less than 1% of tumor cells showed positive staining. Samples were more than 1% tumor cells expressed iNOS were considered as high expressing.

For evaluation of PD-L1 expression we used Combined Positive Score (CPS) (PD-L1 IHC 22C3 pharmDx Interpretation Manual – Esophageal Squamous Cell Carcinoma), which is the number of PD-L1 staining cells (tumor cells, lymphocytes, macrophages) divided by the total number of viable tumor cells, multiplied by 100. According to obtained results the samples were divided into low expression (less then 1%) and high expression (more than 1%) groups.

Statistical analysis

The statistical analysis was performed with GraphPad Prism, Version 8.3, software (San Diego, CA, USA). χ^2 and Fisher exact tests (for categorical variables) were used to compare the differences between the expression of CD68 et al and clinicopathological parameters of ESCCs. Spearman's rank correlation method was used to evaluate the correlations between the amounts of different inflammatory cell types in tumor stroma. Survival length was determined from the date of surgery to death or the date of the last clinical attendance. Survival curves were derived using the Kaplan–Meier method, and differences between curves were analyzed using the log-rank test. In all analyses, *P* values ≤ 0.05 were considered statistically significant

Results

TAMs phenotype

We used CD68 as a common macrophage marker, CD163 and CD206 as M2 marker and iNOS as M1 marker. As an additional general macrophage marker, we used PU.1. We selected PU.1 due to it relatively specific macrophage expression and nuclear pattern of staining what generally simplifies the scoring, in contrast CD68, CD163 and CD206 demonstrate diffuse membrane and cytoplasmic staining that may lead to uncertainties in quantification.

We found CD68+, CD163 + and CD206 + TAMs distributed in both tumor stroma and tumor islets. Analyzed cases were divided into groups with high and low number of M2 macrophages as described above. These groups were used to analyze the association with clinicopathological characteristics. For CD206 significant correlation with the histologic grade was established ($p = 0.034$) ($P > 0.05$ Table 1). No significant correlations were found between CD68+, CD163 + or PU.1 + and clinical characteristics.

Table 1
Clinicopathological characteristics and TAM markers in ESCC

	CD68			CD163			CD206			PU.1			iNOS		
	high	low	p	high	low	p	high	low	p	high	low	p	high	low	p
Stage	12	13	> 0,999	9	16	0,769	13	12	> 0,999	13	12	0,401	1	24	**0,044***
I-II	11	12		10	13		12	11		9	14		6	17	
III-IV															
Nodal status	11	14	0,773	9	16	0,769	13	12	> 0,999	12	13	0,779	2	23	0,237
N-	12	11		10	13		12	11		10	13		5	18	
N+															
Histologic grade	18	20	> 0,999	14	24	0,487	23	15	**0,034***	17	21	> 0,999	6	32	> 0,999
G1/2	5	5		5	5		2	8		5	5		1	9	
G3															
Age	13	13	0,779	10	16	> 0,999	12	14	0,401	10	16	0,384	6	20	0,106
≤ 60	10	12		9	13		13	9		12	10		1	21	
> 60															
Gender	17	19	> 0,999	16	20	0,316	17	19	0,324	16	20	0,751	6	30	0,662
male	6	6		3	9		8	4		6	6		1	11	
female															

* Statistically significant

Further we demonstrate that none of the samples contained iNOS + type 1 macrophages. Though iNOS expression in tumor cells was detected in 12 out of 48 samples in 5 cases this expression was in less than 1% of cells. Expression of iNOS correlated with the disease stage (p = 0.044).

Tumor infiltrating lymphocytes phenotype

For tumor infiltrating T-cells the situation was similar to that with macrophage. In nearly all samples (98%) CD3 + cells were detected within tumor stroma and tumor islets, CD8 + cells were detected in 96% of cases. For the analysis of correlation with clinical data the same approach as for macrophages was used. Samples were divided into high and low density TIL groups according to positive cell count and evaluated possible correlations with clinicopathological parameters, including age, gender, histological grade, nodal status, and clinical stages (Table 2).

Table 2
Clinicopathological characteristics and TIL markers in ESCC.

	CD3			CD8			FOXP3		
	high	low	p	high	low	p	high	low	p
Stage	18	7	0,543	11	14	0,383	10	15	> 0,999
I-II	14	9		7	16		9	14	
III-IV									
Nodal status	18	7	0,543	9	16	> 0,999	11	14	0,566
N-	14	9		9	14		8	15	
N+									
Histologic grade	25	13	> 0,999	14	24	> 0,999	16	22	0,719
G1/2	7	3		4	6		3	7	
G3									
Age	16	10	0,5421	8	18	0,3748	5	21	**0,003***
≤ 60	16	6		10	12		14	8	
> 60									
Gender	25	11	0,4998	13	23	0,7429	11	25	**0,041***
male	7	5		5	7		8	4	
female									
* Statistically significant									

Statistically significant correlation was found solely for FOXP3+, that correlated with the age and gender of patients (p = 0.003 и p = 0.041 respectively).

Programmed death ligand 1 (PD-L1) is a ligand for the inhibitory programmed cell death protein 1 (PD-1), which are targeted by several anti-PD-1 and PD-L1 drugs for a variety of human cancers including metastatic squamous cell carcinoma of the esophagus. In our study group 27% of samples were PD-L1-negative, 62,5% with Combined Positive Score lower than 1% (including PD-L1 negative samples). No statistically significant correlations of PD-L1 expression and clinical parameters was found (supplementary table 2).

Survival analysis

To identify markers of potential prognostic significance in the patients with ESCC, the impacts of TAMs and TILs subgroup and other clinicopathological parameters on the prognosis were explored. To establish the prognostic effect of these clinicopathologic characteristics and markers of immune cells, univariate analysis was used (results are presented in Table 3).

Table 3
Statistical analysis of the prognostic value of immune cells of tumor stroma

	Univariate analysis		
	HR	95% CI	P
CD3 (high/low)	0,9181	(0,3718-2,267)	0,8476
CD8 (high/low)	1,106	(0,4737-2,581)	0,8101
FOXP3 (high/low)	0,5407	(0,2339-1,250)	**0,0462***
CD68 (high/low)	0,8473	(0,3739-1,920)	0,6851
CD163 (high/low)	0,4447	(0,1957-1,010)	**0,0456***
CD206 (high/low)	0,9572	(0,4187-2,189)	0,9149
iNOS (high/low)	0,6928	(0,2635-1,821)	0,4929
PU.1 (high/low)	0,9132	(0,4033-2,068)	0,8242
PDL1 (high/low)	0,7251	(0,3171-1,658)	0,4504
* Statistically significant			

We established that increased CD163 + macrophages and FOXP3 + lymphocytes were significantly associated with prolonged overall survival (OS) in ESCC (p = 0.0456 and p = 0.0462 respectively), and the Kaplan-Meier figures are shown in Fig. 1. For none of other markers statistically significant correlation was found.

PU.1 is a new general macrophage marker

There is an urgent need for new macrophage markers suitable for immunohistochemical analysis showing nuclear staining. As such a marker we used PU.1 in this study. It has a nuclear pattern of expression, what makes it easier to evaluate the data and also allow for multiplex analysis together with other macrophage markers.

We performed a correlation analysis of various macrophage markers in esophagus tumor and demonstrated that PU.1 expression strongly correlate with that of CD68 (r = 0.7515, p = .000), CD163 (r = 0.5847, p = 0.000) and CD206 (r = 0.4245, p = 0.003) with the strongest correlation observed for CD68 (Fig. 2).

IHC analysis of macrophage markers on serial tumor sections also demonstrates highly overlapping staining patterns for PU.1 and other macrophage markers (Fig. 3). Strong correlation of PU.1 primarily with CD68 suggests possible usage of this marker as a general macrophage marker for tumor stroma.

Discussion

Tumor immune escape is an important aspect of tumor development that ensures tumor progression. Tumor cells produce soluble factors that modify microenvironment, attract various immune cells and drive their differentiation to immunosuppressive phenotype. In this study using various markers of tumor stroma cells we investigated immunosuppressive phenotype of esophageal squamous cell carcinoma (ESCC) (Fig. 4).

The main cell population we have studied is composed of TAMs. Like other immune effector and regulatory cells, macrophages demonstrate high degree of functional versatility and express different surface markers, and secretable factors(11). The role they play in the tumor immune escape depends on their phenotype. Macrophages can be boldly divided into two main subgroups: “classically activated” or M1, and “alternatively activated” or M2. M1 are pro-inflammatory and are thought to exert antitumor effects through production of IL-12, IL-23, and reactive oxygen and nitrogen species(12). M1 are not considered to be immunosuppressive, however existence of mixed M1/M2 phenotype of TAMs prompted us to analyze M1 marker – iNOS. We found that this marker is not suitable for TAM analysis, since its expression was observed solely in tumor cells in a small number of samples. Interestingly iNOS expression correlated with the stage of disease, high expression was found in tumors of stages III–IV, compared to low expression at stages I-II.

No prognostic value of iNOS was determined. iNOS expression in esophageal cancer is poorly studied. No significant correlation with the clinical parameters of the tumor and iNOS expression was found in the study by Jin et al(13), the absence of expression difference between tumor and normal tissue of esophagus was also reported(14). Our results, however, suggest that deeper investigation of iNOS in esophageal tumors will reveal its diagnostic and/or prognostic value.

M2 are usually considered to be able to suppress antitumor properties of M1 TAMs and modulate tissue remodeling by producing matrix metalloproteinases, transglutaminases and extracellular matrix components(15) and various cytokines and growth factors(16). In most of the tumors TAMs have M2 phenotype that are considered to be tumor supporting ones(17). All tumor associated macrophages independent on their phenotype, seem to express CD68. As subtype-specific marker of M2 CD163 is frequently used(16). However there are several other markers like CD204, CD206 or Stabilin-1 that can be used to detect type 2 macrophages. We examined the relationship between TAM density, and clinical characteristics and outcomes in 48 patients who had undergone resection of esophageal cancer. We demonstrated that out of all markers studied, only CD206 correlated with the histological grade of the tumor. No other correlations were found.

There are contradictory literature data regarding prognostic value of M2 number in the tumor. In most of the cases high number of M2 TAMs correlates with poor prognosis, since these macrophages promote vascularization, invasion and metastasis in many cancer types(18). In our study, the number of CD163 + M2 correlated with a good prognosis of esophageal cancer (HR = 0,4447, p = 0,0456*). In contrast to our results Hu et al demonstrated correlation of stromal CD163 + TAMs with poor prognosis of esophageal cancer, however if CD163 + cells were counted in tumor nests only, as well no difference in patients survival was found in that study(19). This differences in results clearly indicates the importance of the way how and in which areas of the tumor TAMs are analyzed.

Since the total amount of macrophages is a highly important criteria, there is an urgent need for a macrophage marker that allow clear identification of the cell. We selected PU.1 as such a marker. PU.1 is a transcription factor regulating hematopoietic differentiation pathways(20). Upon lineage differentiation and maturation, PU.1 is expressed at varied levels in mature blood cells, with higher levels found in macrophages than B cells(21). In our study expression of PU.1 showed strongest correlation with CD68, and a staining pattern indicating that the cells stained for PU.1 are CD68 positive macrophages. Taking into account nuclear staining PU.1 will be more suitable for precise cell quantification.

In the present study, we also explored the impact of TILs on the clinical significance in ESCC. It was demonstrated that high numbers of TILs is a marker of good prognosis and longer survival in ESCC. Particularly the presence of T cells (CD3+) and T cell subpopulations (e.g., CD4+, CD8+, CD103+) were established to be markers of a good prognosis(22). CD8 + T cells can recognize tumor-associated antigens as major histocompatibility complex (MHC) class I molecules on the cancer cell surface and lyse cancer cells. Therefore, the presence of CD8 + T cells in the tumor is considered as a host immunoreaction and is associated with a better prognosis in a variety of cancers. However opposite results are also reported, where high levels of CD8 + T cells in the tumor are associated with a poor prognosis(23). In our study we found no significant correlations of CD3 + and CD8 + cells and clinical features of the tumor. As well analysis of prognostic value of T-cells in general and cytotoxic T-cells did not reveal statistically significant differences.

Another T-cell type that has diagnostic and prognostic value in different types of cancer is regulatory T-cells, expressing FOXP3. FOXP3 is a member of the forkhead/winged-helix family of transcription factors that is critically involved in the development and function of Tregs(24). Several studies demonstrate that FOXP3 + Tregs infiltrating tumor suppress CD8 + T cells to maintain immunological tolerance, associates with advanced tumor growth and poor prognosis in several types of malignant tumors(25–27). In contrast, other studies have shown that tumor FOXP3 expression is a favorable prognostic factor for breast cancer(28, 29). In the case of esophageal cancer high numbers of FOXP3 + cells was reported to be an indicator of poor(30) as well as good(31) prognosis. In our study we demonstrated that high number of FOXP3 + cells is associated with good prognosis in the analysis of overall survival (HR = 0.5407, p = 0.0462).

Conclusion

In conclusion, data we obtained are in a good agreement with a number of studies, indicating that TAMs and TILs may provide important diagnostic and prognostic information for esophageal squamous cell carcinoma. However, discrepancies found suggest that there is a need for a general agreement on the methodology of stroma cells evaluation and specifically macrophage counting. As well usage of a nuclear marker for macrophage identification can be recommended what will facilitate stromal cells identification.

Declarations

Ethics approval

The Institutional Review Board of N.N. Blokhin Russian Cancer Research Center approved the project (approval date 09/2018) and all patients, who were involved in the study, gave written informed consents that their samples could be used for research purposes. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki. Data were analyzed anonymously. All potential participants who declined to participate or otherwise did not participate were eligible for treatment (if applicable) and were not disadvantaged in any other way by not participating in the study.

Consent for publication

Not applicable

Availability of data and materials

All data generated or analysed during this study are included in this published article and its supplementary information files.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by the Russian Foundation for Basic Research, grant number 18-29-09069.

Authors' contributions

Dr. O. Kovaleva – analyzed data, writing – original draft preparation

D. Samoilova, M. Rashidova, V. Mochalnikova – performed experiments

Dr. A. Gratchev – supervision, study conceptualization, writing - review and editing

Acknowledgments

We would like to acknowledge the contribution of Dr. Irina Zborovskaya, who contributed to the design of this study.

References

1. Gratchev A, Kzhyshkowska J, Kothe K, Muller-Molinet I, Kannookadan S, Utikal J, et al. Mphi1 and Mphi2 can be re-polarized by Th2 or Th1 cytokines, respectively, and respond to exogenous danger signals. Immunobiology. 2006;211(6–8):473–86.
2. Gratchev A, Guillot P, Hakiy N, Politz O, Orfanos CE, Schledzewski K, et al. Alternatively activated macrophages differentially express fibronectin and its splice variants and the extracellular matrix protein betaIG-H3. Scand J Immunol. 2001;53(4):386–92.

3. Biswas SK, Mantovani A. Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm. Nature immunology. 2010;11(10):889–96.
4. Wynn TA, Chawla A, Pollard JW. Macrophage biology in development, homeostasis and disease. Nature. 2013;496(7446):445–55.
5. Shigeoka M, Urakawa N, Nakamura T, Nishio M, Watajima T, Kuroda D, et al. Tumor associated macrophage expressing CD204 is associated with tumor aggressiveness of esophageal squamous cell carcinoma. Cancer Sci. 2013;104(8):1112–9.
6. Ortegel JW, Staren ED, Faber LP, Warren WH, Braun DP. Modulation of tumor-infiltrating lymphocyte cytolytic activity against human non-small cell lung cancer. Lung Cancer. 2002;36(1):17–25.
7. Ha TY. The role of regulatory T cells in cancer. Immune network. 2009;9(6):209–35.
8. von Boehmer H, Daniel C. Therapeutic opportunities for manipulating T(Reg) cells in autoimmunity and cancer. Nat Rev Drug Discov. 2013;12(1):51–63.
9. Jiang Y, Lo AWI, Wong A, Chen W, Wang Y, Lin L, et al. Prognostic significance of tumor-infiltrating immune cells and PD-L1 expression in esophageal squamous cell carcinoma. Oncotarget. 2017;8(18):30175–89.
10. Chen K, Cheng G, Zhang F, Zhang N, Li D, Jin J, et al. Prognostic significance of programmed death-1 and programmed death-ligand 1 expression in patients with esophageal squamous cell carcinoma. Oncotarget. 2016;7(21):30772–80.
11. Gratchev A. TGF-beta signalling in tumour associated macrophages. Immunobiology. 2017;222(1):75–81.
12. Duray A, Demoulin S, Hubert P, Delvenne P, Saussez S. Immune suppression in head and neck cancers: a review. Clin Dev Immunol. 2010;2010:701657.
13. Jin Y, Zhang W, Liu B. [Abnormal expression of p53, Ki67 and iNOS in human esophageal carcinoma in situ and pre-malignant lesions]. Zhonghua zhong liu za zhi [Chinese journal of oncology]. 2001;23(2):129–31.
14. Barani R, Motalleb G, Maghsoudi H. Evaluation of iNOS Expression in Esophageal Cancer Patients. Gastrointest Tumors. 2016;3(1):44–58.
15. Gratchev A, Kzhyshkowska J, Utikal J, Goerdt S. Interleukin-4 and dexamethasone counterregulate extracellular matrix remodelling and phagocytosis in type-2 macrophages. Scand J Immunol. 2005;61(1):10–7.
16. Kovaleva OV, Samoilova DV, Shitova MS, Gratchev A. Tumor Associated Macrophages in Kidney Cancer. Anal Cell Pathol (Amst). 2016;2016:9307549.
17. Hao NB, Lu MH, Fan YH, Cao YL, Zhang ZR, Yang SM. Macrophages in tumor microenvironments and the progression of tumors. Clin Dev Immunol. 2012;2012:948098.
18. Mantovani A, Sozzani S, Locati M, Allavena P, Sica A. Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. Trends Immunol. 2002;23(11):549–55.
19. Hu JM, Liu K, Liu JH, Jiang XL, Wang XL, Chen YZ, et al. CD163 as a marker of M2 macrophage, contribute to predicte aggressiveness and prognosis of Kazakh esophageal squamous cell carcinoma. Oncotarget. 2017;8(13):21526–38.
20. Mak KS, Funnell AP, Pearson RC, Crossley M. PU.1 and Haematopoietic Cell Fate: Dosage Matters. Int J Cell Biol. 2011;2011:808524.
21. Klemsz MJ, McKercher SR, Celada A, Van Beveren C, Maki RA. The macrophage and B cell-specific transcription factor PU.1 is related to the ets oncogene. Cell. 1990;61(1):113–24.
22. Schumacher K, Haensch W, Roefzaad C, Schlag PM. Prognostic significance of activated CD8(+) T cell infiltrations within esophageal carcinomas. Cancer Res. 2001;61(10):3932–6.
23. Tian C, Lu S, Fan Q, Zhang W, Jiao S, Zhao X, et al. Prognostic significance of tumor-infiltrating CD8(+) or CD3(+) T lymphocytes and interleukin-2 expression in radically resected non-small cell lung cancer. Chin Med J (Engl). 2015;128(1):105–10.
24. Sakaguchi S, Miyara M, Costantino CM, Hafler DA. FOXP3 + regulatory T cells in the human immune system. Nature reviews Immunology. 2010;10(7):490–500.
25. Curiel TJ, Coukos G, Zou L, Alvarez X, Cheng P, Mottram P, et al. Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. Nature medicine. 2004;10(9):942–9.
26. Hiraoka N, Onozato K, Kosuge T, Hirohashi S. Prevalence of FOXP3 + regulatory T cells increases during the progression of pancreatic ductal adenocarcinoma and its premalignant lesions. Clinical cancer research: an official journal of the American Association for Cancer Research. 2006;12(18):5423–34.

27. Kobayashi N, Hiraoka N, Yamagami W, Ojima H, Kanai Y, Kosuge T, et al. FOXP3 + regulatory T cells affect the development and progression of hepatocarcinogenesis. Clinical cancer research: an official journal of the American Association for Cancer Research. 2007;13(3):902–11.
28. Ladoire S, Arnould L, Mignot G, Coudert B, Rebe C, Chalmin F, et al. Presence of Foxp3 expression in tumor cells predicts better survival in HER2-overexpressing breast cancer patients treated with neoadjuvant chemotherapy. Breast Cancer Res Treat. 2011;125(1):65–72.
29. Zuo T, Liu R, Zhang H, Chang X, Liu Y, Wang L, et al. FOXP3 is a novel transcriptional repressor for the breast cancer oncogene SKP2. J Clin Investig. 2007;117(12):3765–73.
30. Wang G, Liu G, Liu Y, Li X, Su Z. FOXP3 expression in esophageal cancer cells is associated with poor prognosis in esophageal cancer. Hepatogastroenterology. 2012;59(119):2186–91.
31. Wada Y, Date Y, Ohike N, Tsukuda G, Asonuma K, Morohoshi T, et al. Clinicopathological Significance of FOXP3 Expression in EsophagealSquamous Cell Carcinoma. The Showa University Journal of Medical Sciences. 2013;25(2):133–42.

Figures

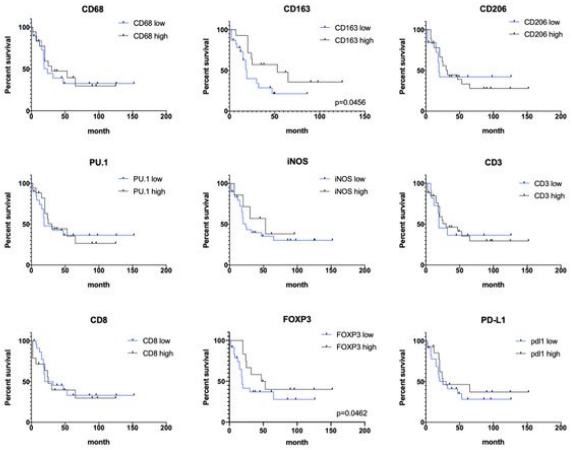

Figure 1

Figure 1

Kaplan-Meier curves of overall survival (OS) in esophageal squamous cell carcinoma (ESCC) based on TILs and TAMs.

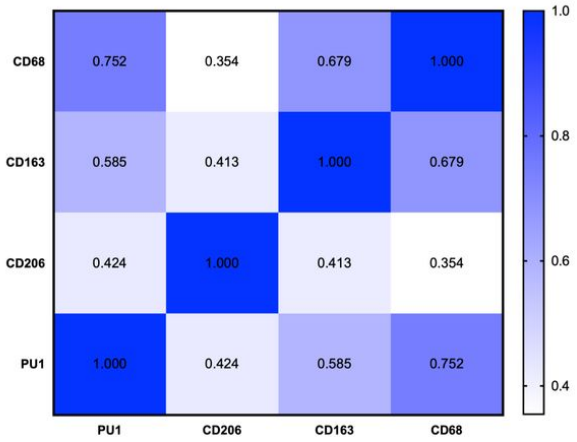

Figure 2

Figure 2

Spearman's rank correlation coefficient for macrophage markers.

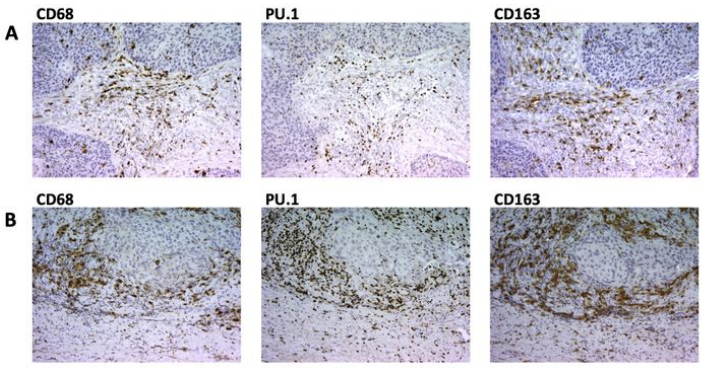

Figure 3

Figure 3

Immunohistochemical analysis of CD68, PU.1 and CD163 on serial tissue sections. Magnification 400x (A) and 1000x (B).

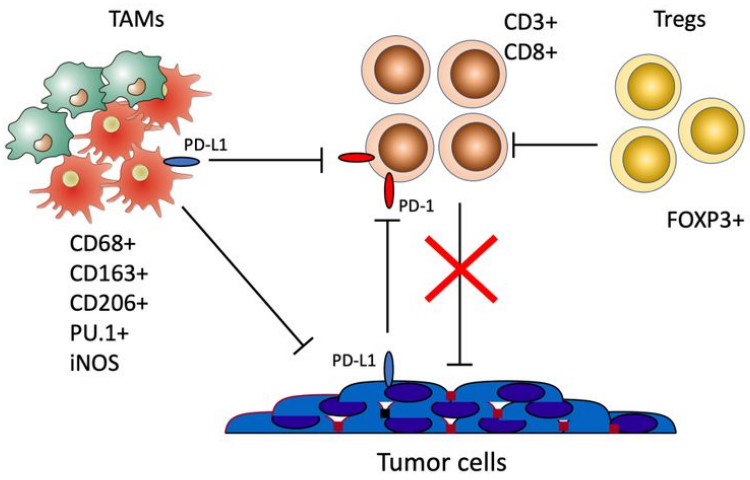

Figure 4

Figure 4

Immunosuppressive cells of esophageal squamous cell carcinoma stroma.

Supplementary Files

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