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RESEARCH ARTICLE

Circulating $CD14^{+}HLA\text{-}DR^{-/low}$ myeloid-derived suppressor cell is an indicator of poor prognosis in patients with ESCC

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Abstract Accumulating evidences demonstrate that a population of suppressive cells known as myeloid-derived suppressor cells (MDSCs) is key immune modulators which suppress antitumor immunity. In this study, we found that the level of circulating $CD14^{+}HLA\text{-}DR^{-/low}$ cells in patients was significantly higher than that of healthy donors and was correlated with tumor burden, lymph node metastasis, and tumor, node, and metastasis (TNM) clinical stage. More importantly, we for the first time find the level of $CD14^{+}HLA\text{-}DR^{-/low}$ is a biological indicator of poor prognosis through the analysis of 3-year overall survival. Furthermore, we evidenced that the proportion of $CD14^{+}HLA\text{-}DR^{-/low}$ cells in the tumor metastatic tumor-draining lymph nodes (TDLNs) was notably higher compared to tumor-free TDLNs. Additionally, $CD14^{+}HLA\text{-}DR^{-/low}$ cells from esophageal squamous cell carcinoma (ESCC) patients expressed dramatically increased programmed death ligand 1 (PD-L1) comparing to that from healthy control. Subsequently, blocking PD-L1 pathway by antibody could effectively reverse the suppressive effect on autologous T cell proliferation mediated by $CD14^{+}HLA\text{-}DR^{-/low}$ cells in vitro. In conclusion, our data revealed $CD14^{+}HLA\text{-}DR^{-/low}$ MDSCs which increase in ESCC patients is a novel poor prognostic indicator and may exert immunosuppressive properties through PD-L1/PD-1 pathway.

Keywords MDSC · PD-L1 · Esophageal squamous cell carcinoma · Prognostic marker

Abbreviations

MDSCs	Myeloid-derived suppressor cells
ESCC	Esophageal squamous cell carcinoma
PD-L1	Programmed death ligand 1
TDLNs	Tumor-draining lymph nodes
PBMCs	Peripheral blood mononuclear cells
ARG-1	Arginase-1
ROS	Reactive oxygen species
iNOS	Inducible nitric oxide synthase
TGF-β	Transforming growth factor β
Tregs	Regulatory T cells
PIR-B	Paired immunoglobulin-like receptors B
CFSE	Carboxyfluorescein succinimidyl ester

Introduction

Patients with malignancy are known to be immunologically depressed. Tumor cells actively induce the anergy and exhaustion of effector T cells and promote the expansion of negative regulatory immunocytes, which downregulate antitumor immunity [1–3]. Myeloid-derived suppressor cells (MDSCs) represent a heterogeneous population of the myeloid cell

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lineage which comprised myeloid progenitor cells and immature myeloid cells (IMCs). Normally, IMCs generated in the bone marrow quickly differentiate into mature granulocytes and macrophages or dendritic cells (DCs). In pathological conditions such as cancer [4–6], infectious diseases [7, 8], stress [9], chemotherapy [10], or some autoimmunity [11, 12], a partial blockage in the differentiation of IMCs into mature myeloid cells results in an expansion of the MDSC population.

In mice, MDSCs are defined based on the expression of Gr1 and CD11b. Granulocytic MDSCs have a $CD11b^{+}Ly6G^{+}Ly6C^{low}$ phenotype, and monocytic MDSCs are $CD11b^{+}Ly6G^{-}Ly6C^{high}$ [13, 14]. In humans, MDSCs are generally agreed to be $CD14^{-}CD11b^{+}$ cells, or more precisely, cells that express the common myeloid marker CD33 but lack the expression of the markers of mature myeloid and lymphoid cells as well as the MHC class II molecule HLA-DR [4, 15]. In renal cancer patients, a $CD11b^{-}CD15^{+}CD14^{-}$ population was shown to have characteristics of MDSCs [16]. In several recent studies, a new subset of $CD14^{+}HLA\text{-}DR^{-/low}$ cells was identified in the peripheral blood or tumor tissue from patients with melanoma [17, 18], hepatocellular carcinoma [19], ovarian carcinoma [20], prostate cancer [21], and carcinoma of the head and neck [22], supporting the concept of MDSC heterogeneity and plasticity. This heterogeneity suggests that there may be no specific markers that precisely define MDSCs and that suppressive activity is the ultimate defining characteristic [23]. Therefore, different types and different stages of human tumors might induce different subtypes of MDSCs [17, 19]. As a newly identified MDSC population, $CD14^{+}HLA\text{-}DR^{-/low}$ cells has been the focus of attention for their suppressive activity in antitumor immunity and clinical relevance.

Esophageal carcinoma constitutes the sixth cause of cancer-related deaths worldwide [24]. Despite the ongoing research and clinical intervention in esophageal squamous cell carcinoma (ESCC), the long-term survival of patients remains poor. Surgical treatment alone for locally advanced disease results in a 5-year survival rate of only 20–25 %. Although the addition of combined modality strategies of preoperative or postoperative chemoradiotherapy was reported to improve the 5-year survival rates by 10–15 % [25, 26], the therapeutic toxicity remains to be a big problem. Therefore, development of new therapy modalities for ESCC is in dire need. Further understanding of the mechanism of suppressive activity mediated by negative regulatory immunocytes such as MDSCs might provide a new approach to the development of new immune therapies for ESCC.

In this study, we intend to detect the levels of $CD14^{+}HLA\text{-}DR^{-/low}$ cells in ESCC patients and analyze its clinical significance.

Material and methods

Ethics statement

This study was approved by the ethics committee of the First Affiliated Hospital of Soochow University (Suzhou, China) for clinical investigation, and written informed consent was obtained from patients or their relatives before enrollment.

Patients and specimens

Peripheral blood was obtained from 78 patients with pathologically and clinically confirmed ESCC from June 2010 to December 2010. The patients had never been diagnosed as malignancy before and had not received antitumor drugs, radiotherapy, or surgery before blood was drawn. All the patients had undergone surgical treatment as initial therapy, with 76 cases that received esophagectomy, and two cases with unresectable tumor that received exploratory thoracotomy and gastrostomy. Each patient was classified on the basis of the tumor, node, and metastasis (TNM) classifications of malignant tumors by the International Union Against Cancer (UICC) after operation. Patient characteristics are summarized in Table 1. Blood samples from 12 randomly selected patients who had accepted esophagectomy were resurveyed 30 days after surgery and before postoperative chemotherapy or radiotherapy. Survival status and postoperative data were gained from telephone contacts and hospital records, respectively. The survival time was measured as the interval between date of surgical treatment and date of death for any cause. Thirty-

Table 1 Clinicopathological characteristics of patients with ESCC tested in the present study

Characteristic	Value
Total no. patients (male/female)	78 (58/20)
Age, mean (range) (years)	62.4±7.33 (46–77)
Histopathological classification	
Squamous cell carcinomas	78
TNM classification	
T1–2	33
T3–4	45
N0	44
N1–3	34
M0	78
M1	0
Stage	
I	15
II	30
III	33
IV	0

five age- and gender-matched healthy volunteers (62.63± 9.38 years; female/male=8/27) were recruited as a control group. We defined as healthy donors those who had never received any diagnosis of malignancy and showed no abnormalities in complete blood cell counts, C-reactive proteins, erythrocyte sedimentation rate, liver function tests, or chest X-rays in a medical checkup.

Flow cytometry analysis

Peripheral blood samples were stained with fluorochrome-conjugated mAbs and then analyzed by multicolor flow cytometry (FC500, Beckman Coulter, France). Isotype-matched antibodies were used as controls. The mAbs used in this study are listed as follows: fluorescein isothiocyanate HLA-DR-ECD, CD14-PE, CD11b-FITC, CD33-PC5 CD80-PE, CD86-PE, PD-L1-PE, B7-H3-PE (BioLegend, USA), and B7-H4-PE (Santa Cruz Biotechnology, USA). Respective immunoglobulin G (IgG) isotype-matched controls (BioLegend, USA) were used as negative controls. Data were analyzed with CXP software (Beckman Coulter).

Fresh tumor-draining lymph nodes (TDLNs) of ESCC patients were collected during esophagectomy (two nodes for one case, one node is nearby the tumor site and the other one is far from the tumor site). The nodes were cut apart along macroaxis. Specimens were gently minced and passed through a cell strainer to achieve a single-cell suspension. Then, the frequency of $CD14^+HLA\text{-}DR^{-/low}$ cells was detected. According to the result of pathological examination, tumor metastatic lymph nodes and autologous tumor-free lymph nodes from eight patients were enrolled in this research.

The frequency of $CD14^+HLA\text{-}DR^{-/low}$ cells (%) on total single cells from peripheral blood mononuclear cell (PBMC) or TDLN tissues=CD14 (%)×$CD14^+HLA\text{-}DR^{-/low}$ (%).

Cell isolation and sorting

PBMCs were separated from fresh blood samples (50 mL) by Ficoll density gradient centrifugation. The $CD3^+$ T cells were magnetically enriched by using the CD3-negative isolation kit (STEMCELL, Canada) from PBMC, and the purity >95 % is selected for the following study. The purified $CD14^+HLA\text{-}DR^{-/low}$ and $CD14^+HLA\text{-}DR^{high}$ cells were sorted by using the Beckman Coulter MoFlo^{TM} cell sorting system (Beckman Coulter, France), and the purity >90 % was chosen for the following experiment.

Carboxyfluorescein succinimidyl ester-based suppression assay

$CD3^+$ T cells were labeled with carboxyfluorescein succinimidyl ester (CFSE) (Invitrogen, USA) according to the manufacturer's instructions. The labeled T cells (1×10^5/well) were incubated with sorted $CD14^+HLA\text{-}DR^{low/-}$ cells (2.5×10^4/well) from the same patient in 96-well plates in the presence of CD3/CD28 mAbs immunobeads (Miltenyi Biotec, German). T cells alone were used as control. After 72 h, cells were harvested for flow analysis. For blocking experiments, we intervened programmed death ligand 1 (PD-L1) by using a blocking antibody (1 μg/mL, eBioscience, USA, clone MIH1) in the co-culture of $CD14^+HLA\text{-}DR^{-/low}$ cells and autologous T cells.

Statistical analysis

The data is reported as mean±SD. Flow cytometry data between different groups were compared by two-sample *t* test (Mann-Whitney test). Paired *t* test was used to compare the frequency of circulating $CD14^+HLA\text{-}DR^{-/low}$ cells pre- and post-operation. Levels of $CD14^+HLA\text{-}DR^{-/low}$ cells in tumor metastatic and tumor-free TDLNs were also compared by paired *t* test. One-way analysis of variance was used to test of differences between more than two groups. Fisher's exact test was performed to analyze the clinical significance of circulating $CD14^+HLA\text{-}DR^{-/low}$ cells. Kaplan-Meier survival curves were created for descriptive purposes and compared by log-rank test. All analyses were carried out by using the GraphPad Prism 5.0 software (version 5.01). *p values <0.05 and **p values <0.01 were considered to be statistically significant.

Results

The $CD14^+HLA\text{-}DR^{-/low}$ cells in the peripheral blood were significantly increased in patients with ESCC

We firstly examined the levels of two subpopulations of MDSCs marked as $CD14^+HLA\text{-}DR^{-/low}$ cells and $CD11b^+CD33^+CD14^-HLA\text{-}DR^{-/low}$ cells presented in the peripheral blood of patients with ESCC and healthy donors. The percentage of circulating $CD14^+HLA\text{-}DR^{-/low}$ cells in ESCC patients was significantly higher than that of healthy donors (1.85±1.59 vs 0.611±0.58 %, $p<0.001$; as shown in Fig. 1a, b). However, $CD11b^+CD33^+CD14^-HLA\text{-}DR^{-/low}$ cells were not different between patients and healthy donors (data not shown). Our data indicated that $CD14^+HLA\text{-}DR^{-/low}$ cells but not $CD11b^+CD33^+CD14^-HLA\text{-}DR^{-/low}$ cells significantly increase in patients with ESCC. Additionally, we found the frequency of circulating $CD14^+HLA\text{-}DR^{-/low}$ cells significantly decreased with the removal of tumor mass (1.81±0.89 vs 1.01±0.42 %, $p=0.0135$, as shown in Fig. 1c). Maybe, our data indicated that tumor burden induced the accumulation of circulating $CD14^+HLA\text{-}DR^{-/low}$ cells in patient with ESCC.

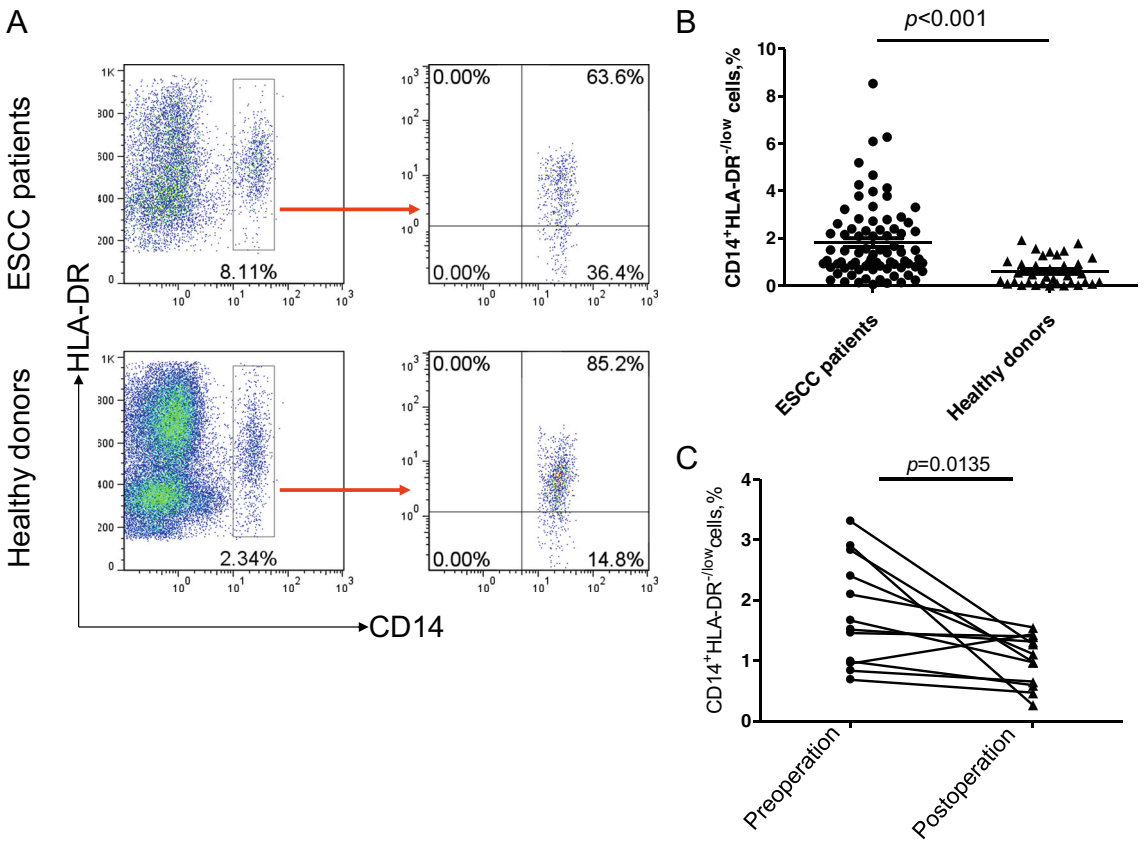

Fig. 1 The frequency of circulating CD14$^+$HLA-DR$^{-/low}$ cells in PBMC from healthy control and ESCC patients. **a** Flow cytometry analysis of circulating CD14$^+$HLA-DR$^{-/low}$ cells in ESCC patients and healthy donors. Representative data from one ESCC patient and one healthy donor. For the CD14$^+$HLA-DR$^{-/low}$ cells, PBMCs were stained with HLA-DR-ECD and CD14-PE mAb. Flow cytometry analysis was performed with gates set on PBMC, and the results were presented as a percentage of CD14$^+$HLA-DR$^{-/low}$ cells in PBMC. **b** Summary of frequency of circulating CD14$^+$HLA-DR$^{-/low}$ cells in PBMC obtained from ESCC patients (n=78) and healthy donors (n=35). *Lines* indicate mean values. **c** Comparison of levels of CD14$^+$HLA-DR$^{-/low}$ in PBMC from ESCC patients before and after operation. Student t test (paired test) was performed, and each *dot* represents one case (n=12)

The frequency of circulating CD14$^+$HLA-DR$^{-/low}$ cells was correlated with tumor burden, lymph node metastasis, and tumor stage

Next, we assessed whether the level of circulating CD14$^+$HLA-DR$^{-/low}$ cells was associated with tumor progression. Through clinical data analysis, we found that the increase in circulating CD14$^+$HLA-DR$^{-/low}$ cells was correlated with lymph node metastasis (p=0.0093) and later clinical stage (p=0.0486), but not with other characteristics, such as age, gender, and pathological differentiation (as shown in Table 2). These results suggested that circulating CD14$^+$HLA-DR$^{-/low}$ cells might be involved in tumor metastasis and progression.

CD14$^+$HLA-DR$^{-/low}$ cells accumulated in the tumor metastatic lymph node

Since CD14$^+$HLA-DR$^{-/low}$ cells were correlated with lymph node metastasis, we analyzed the frequency of CD14$^+$HLA-DR$^{-/low}$ cells in the tumor metastatic TDLNs and tumor-free TDLNs of the same patient with ESCC. We found a significant increase in the percentage of CD14$^+$HLA-DR$^{-/low}$ cells in the tumor metastatic lymph nodes compared with tumor-free nodes (0.81±0.78 vs 0.20±0.21 %, p=0.0453, Fig. 2).

CD14$^+$HLA-DR$^{-/low}$ cells suppressed T cell proliferation depending upon PD-L1/PD-1 signal

Co-stimulatory and co-inhibitory molecules, particularly B7 superfamily members, play an important role in regulating T cell responses. To evaluate the potential mechanism of CD14$^+$HLA-DR$^{-/low}$ cells in suppressing T proliferation, we examined the surface expression of B7 family molecules on peripheral circulating CD14$^+$HLA-DR$^{-/low}$ cells from patients with ESCC and healthy donors. Very interestingly, only co-inhibitory molecule PD-L1 was significantly highly expressed on CD14$^+$HLA-DR$^{-/low}$ cells (Fig. 3a, b). Therefore, we estimated that CD14$^+$HLA-DR$^{-/low}$ cells suppressed T cell proliferation depending on PD-L1. We blocked PD-L1 pathway

Table 2 Relationship between circulating $CD14^+HLA\text{-}DR^{-/low}$ cells and clinical characteristics in patients with ESCC

Characteristics	Number	$CD14^+HLA\text{-}DR^{-/low}$ cell (%)	*p* value
Gender			
Male	58	1.90±0.23	0.6405
Female	20	1.71±0.25	
Age (years)			
<60	28	1.69±0.20	0.2305
≥60	50	2.14±0.34	
T factor			
T1~2	33	1.57±0.25	0.1758
T3~4	45	2.06±0.25	
N factor			
0	44	1.45±0.18	0.0093
1–3	34	2.38±0.33	
Differentiation			
Well	10	1.16±0.25	0.2831
Moderate	40	2.02±0.30	
Poor	28	1.72±0.22	
Stage			
I~II	45	1.55±0.21	0.0486
III~IV	33	2.27±0.30	

using a special antibody against PD-L1 in the co-culture of $CD14^+HLA\text{-}DR^{-/low}$ cells and autologous T cells. We found that T cell proliferative suppression was effectively reversed by blocking PD-L1 pathway (Fig. 3c).

The high level of circulating $CD14^+HLA\text{-}DR^{-/low}$ cells serves as a poor prognostic marker in patients with ESCC

The patients were followed up for 3 years until December 2013; four cases were lost to follow-up, and the 3-year overall survival rate was 56.41 %. Two cases died within 30 days after surgery (20 and 21 days, respectively) and were excluded in this survival analysis. ROC analysis indicated that an optimal cutoff point for the frequency of circulating $CD14^+HLA\text{-}DR^{-/low}$ cells in predicting the 3-year overall survival is 2.38 %. Based on this cutoff value, the patients were divided into two groups, with 54 cases (71 %) in $CD14^+HLA\text{-}DR^{-/low}$ cells high level group and 22 cases (29 %) in $CD14^+HLA\text{-}DR^{-/low}$ low level group. Kaplan-Meier survival curves showed that patients of the high level group had a significantly poorer outcome comparing to the low level group (log-rank test, $p=0.0017$) (Fig. 4).

Discussion

Accumulating evidences demonstrate that MDSCs are key immune modulators which suppress lymphocyte responses in tumor-bearing animals and cancer patients. MDSCs were firstly described in patients with cancer as natural suppressor cells in 1980s [27–29]. Subsequently, this immature, myeloid-derived, and suppressive population was detected in tumor-bearing mice and was named as immature myeloid cells (IMCs) or myeloid suppressor cells (MSCs). In 2007, these cells were suggested to be named as myeloid-derived suppressor cells (MDSCs) for its myeloid origin and biological function [30]. The characteristics and biological functions of MDSCs have always been in the center of concerns.

It has been proved that MDSCs represent a heterogeneous population of immunosuppressive cells. In the past several years, a new subset of $CD14^+$ $HLA\text{-}DR^{-/low}$ cells was identified in the peripheral blood or tumor tissue from a variety of malignancy patients. In 2006, one group described a population of $CD14^+CD16^+HLA\text{-}DR^-$ cells increased in ascites of patients with epithelial ovarian cancer [20]. In 2007, Filipazzi and colleagues identified a subset of $CD14^+HLA\text{-}DR^{-/low}$ myeloid suppressor cells in peripheral blood of melanoma patients with modulation by a granulocyte-macrophage colony-stimulation factor-based antitumor vaccine [17]. In 2008, Hoechst and colleagues observed $CD14^+HLA\text{-}DR^{-/low}$ cells in the blood and tumor of hepatocellular carcinoma patients [19]. Furthermore, they proposed that this new type of MDSCs exerted their immunosuppressive function through the induction of regulatory T cells (Tregs) in co-cultured $CD4^+$ T cells [19]. Soon, several studies identified the $CD14^+$ $HLA\text{-}DR^{-/low}$ cells in the peripheral blood or tumor from patients with ovarian carcinoma [20], prostate cancer [21], and carcinoma of the head and neck [22], respectively.

In this paper, we also observed a significant increase of $CD14^+HLA\text{-}DR^{-/low}$ cells rather than $CD11b^+CD33^+CD14^-HLA\text{-}DR^{-/low}$ cells in the peripheral blood of patients with ESCC compared with healthy donors. Additionally, the levels of circulating $CD14^+HLA\text{-}DR^{-/low}$ cells were notably correlated with lymph node metastasis, TNM clinical stage, and 3-year overall survival. Furthermore, in the case-to-case analysis, the levels of circulating $CD14^+HLA\text{-}DR^{-/low}$ cells decreased with the removal of tumor mass by surgical treatment, which indicated that tumor burden induced the accumulation of circulating $CD14^+HLA\text{-}DR^{-/low}$ cells in patient with ESCC. Our data suggested that $CD14^+HLA\text{-}DR^{-/low}$ cells really represent an important immunosuppressive component involved in the tumor immune escape of ESCC, and the frequency of circulating $CD14^+HLA\text{-}DR^{-/low}$ cells might provide a novel prognostic indicator of ESCC.

In fact, the survival analysis of prognostic factors indicated that the differentiation, T factor, N factor, and circulating $CD14^+HLA\text{-}DR^{-/low}$ MDSC levels significantly correlated with prognosis of patients with ESCC ($p<0.05$, respectively, data not shown). However, if differentiation, T factor, N

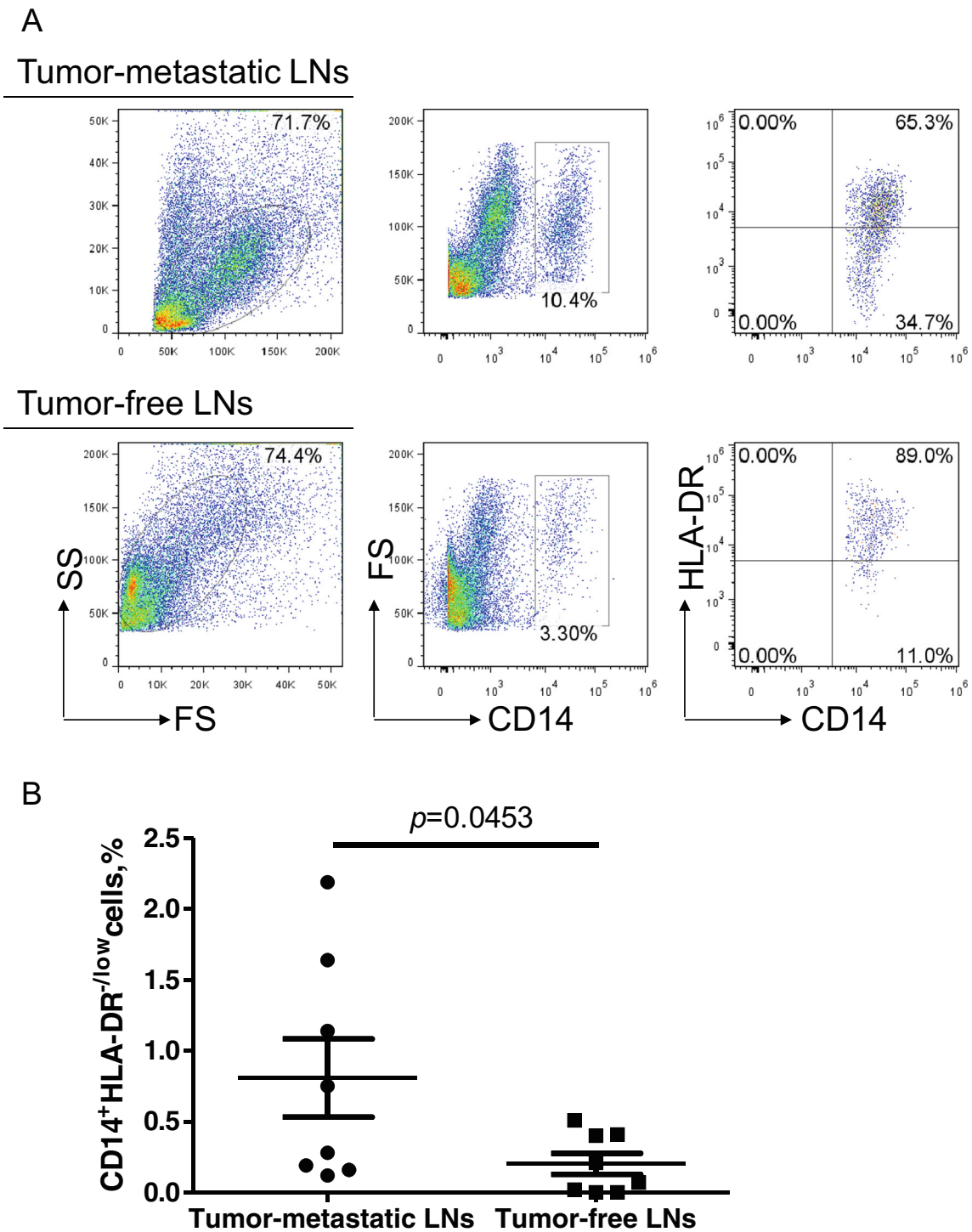

Fig. 2 $CD14^{+}HLA\text{-}DR^{-/low}$ cells increased in the tumor metastatic TDLNs. **a** Flow cytometry analysis of $CD14^{+}HLA\text{-}DR^{-/low}$ cells in the tumor-free lymph nodes and autologous tumor metastatic lymph nodes. Representative data from one tumor-free lymph node and one tumor metastatic lymph node. For the $CD14^{+}HLA\text{-}DR^{-/low}$ cells, PBMCs were stained with HLA-DR-ECD and CD14-PE mAb. **b** Frequency of $CD14^{+}HLA\text{-}DR^{-/low}$ cells in the tumor-free lymph nodes (n=8) and autologous tumor metastatic lymph nodes (n=8). *Lines* indicate mean values

factor, and circulating $CD14^{+}HLA\text{-}DR^{-/low}$ MDSC levels were selected to analyze in a Cox model; Cox multivariate regression analysis indicated only the T factor is an independent risk factor for death (data not shown). That is to say, circulating $CD14^{+}HLA\text{-}DR^{-/low}$ MDSC levels is not an independent prognostic indicator for patients with ESCC.

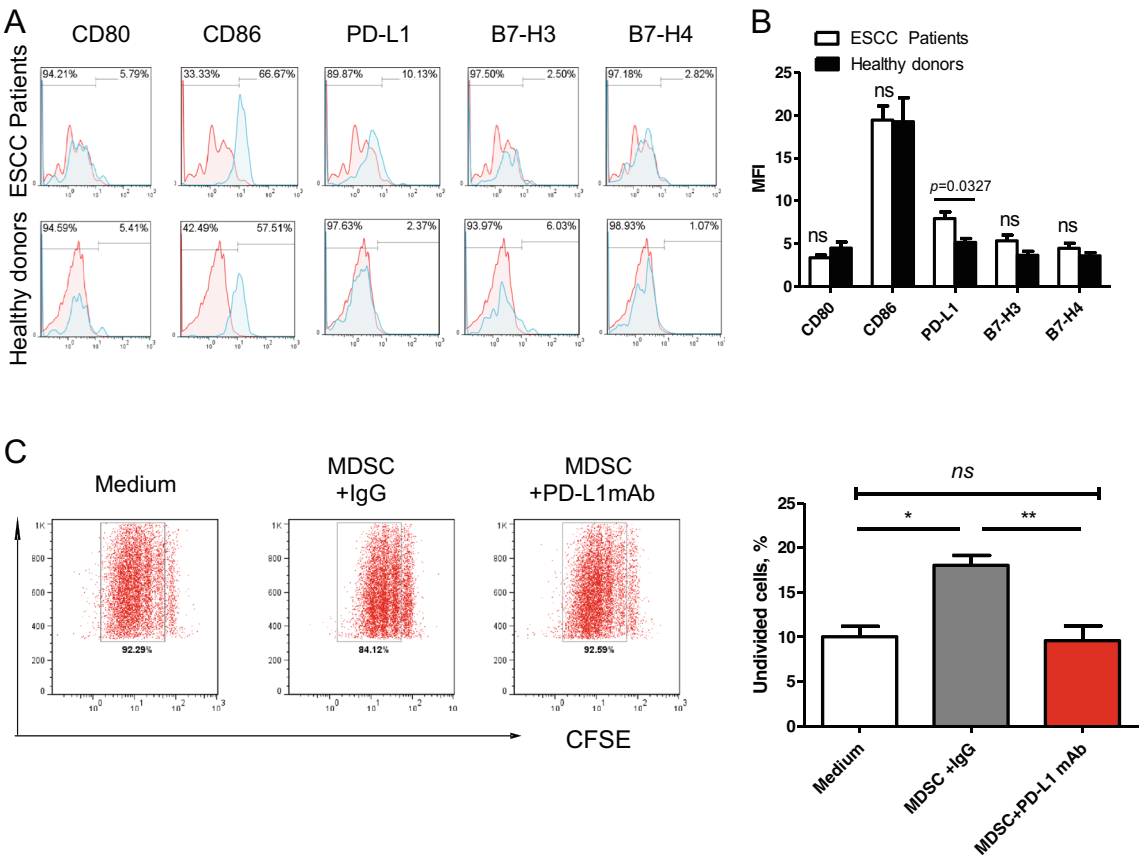

Fig. 3 $CD14^+HLA\text{-}DR^{-/low}$ cells suppress autologous T cell proliferation depending on PD-L1. **a** The expression of co-stimulatory molecules CD80, CD86, PD-L1, B7-H3, and B7-H4 on $CD14^+HLA\text{-}DR^{-/low}$ cells was examined by flow cytometry. Representative data from one ESCC patient and healthy donor. $CD14^+HLA\text{-}DR^{-/low}$ cells were stained with CD80-PE, CD86-PE, PD-L1-PE, B7-H3-PE, and B7-H4-PE mAb. **b** The summary of expression of co-stimulatory molecules CD80, CD86, PD-L1, B7-H3, and B7-H4 on $CD14^+HLA\text{-}DR^{-/low}$ cells from patients with ESCC ($n=15$) and healthy donors ($n=10$). **c** Proliferation of anti-CD3/anti-CD28-stimulated autologous T cells co-cultured with $CD14^+HLA\text{-}DR^{-/low}$ cells in the presence of PD-L1 mAb or IgG was measured by dilution of CFSE staining intensity using flow cytometry. Summary data was list ($n=3$). One-way ANOVA was performed and data are presented as means±SEM. $^{**}p<0.05$; $^{*}p<0.01$; *ns* represents $p>0.05$

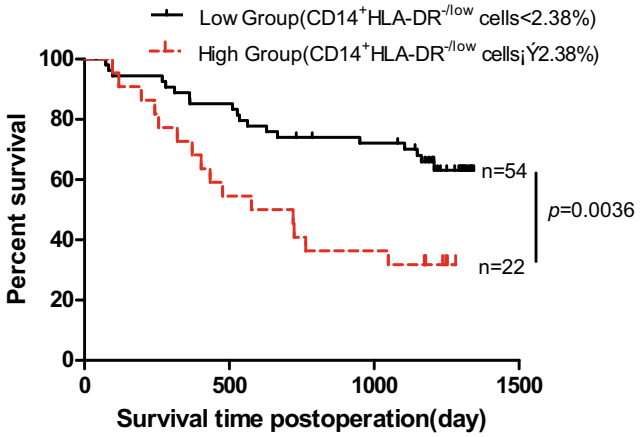

Fig. 4 High level of circulating $CD14^+HLA\text{-}DR^{-/low}$ cells serves as a poor prognostic marker for the survival of patients with ESCC. Kaplan-Meier survival curves for ESCC patients who have received surgical treatment. Kaplan-Meier survival curves showed that patients of $CD14^+HLA\text{-}DR^{-/low}$ cells high level group ($n=22$) had a significantly poorer outcome comparing to $CD14^+HLA\text{-}DR^{-/low}$ cells low level group ($n=54$)

MDSCs also play an important role in tumor metastatic spread. $CD11b^+/Ly6Cmed/Ly6G^+$ cells were identified as key constituents of the premetastatic niche in mammary and melanoma experimental metastasis models [31]. In highly metastatic murine mammary tumors, $Gr\text{-}1^+/CD11b^+$ MDSCs selectively express proteins involved in the c-glutamyl transferase, glutathione synthase pathways, and other pathways involved in platelet aggregation, as well as lipid and amino acid metabolism [32]. Compromised recruitment of MDSCs inhibits tumor growth and pulmonary metastasis of breast cancer mouse model [33]. We found a higher percentage of $CD14^+HLA\text{-}DR^{-/low}$ cells in the tumor metastatic TDLNs compared with the corresponding tumor-free ones. It indicated that $CD14^+HLA\text{-}DR^{-/low}$ cells might contribute to the lymph node metastasis of ESCC.

The mechanisms of immune suppression by MDSCs include nutrient starvation [34] and induction of Tregs [35]. Several key molecules, such as arginase 1 (ARG-1), inducible

nitric oxide synthase (iNOS), and transforming growth factor β (TGF-β) [36], as well as some other immunosuppressive cytokines [37, 38] mediate these suppressive modalities. It is reported recently that monocytic MDSCs are the main source of key immunosuppressive factors such as ARG-1, reactive oxygen species (ROS), and TGF-β1 in neuroblastoma tumor-bearing mice [39]. In addition to their suppressive effects on adaptive immune responses, MDSCs have also been reported to regulate innate immune responses by modulating the cytokine production of macrophages [40]. However, the immune suppression mechanisms of $CD14^+HLA\text{-}DR^{-/low}$ cells remain controversial. For instance, $CD14^+HLA\text{-}DR^{-/low}$ cells in hepatocellular carcinoma patients had high arginase activity, but they did not secrete TGF-β [19], while in melanoma patients, suppressive activity of $CD14^+HLA\text{-}DR^{-/low}$ cells was reported to be mediated by TGF-β rather than arginase [17]. MDSCs are driven by tumor burden and by the diversity of factors produced by the tumor and by host cells in the tumor microenvironment. Thus, the biological functions and phenotype of MDSCs driven by the tumor microenvironment of multiple types of tumors and different stages of the diseases might be various.

It is now clear that MDSCs, though heterogeneous, particularly contribute to the establishment of tumor antigen-specific tolerance and immune escape. To achieve effective antitumor immunity, tumor-induced immunosuppression must be reversed. In mouse models, MDSC depletion or the blockage of their suppressive pathways delays tumor development and growth [41]. The inhibition of certain signaling pathway such as paired immunoglobulin-like receptors B (PIR-B) promoted MDSCs and differentiated and retarded tumor growth of tumor-bearing mice [42]. In humans, several approaches, such as the use of chemotherapeutic agents and induction of the differentiation of MDSCs, have been described for the deletion of MDSCs [5, 43].

To investigate the effect of $CD14^+HLA\text{-}DR^{-/low}$ cells on T responses, we firstly examined the suppressive activity of $CD14^+HLA\text{-}DR^{-/low}$ cells. As expected, $CD14^+HLA\text{-}DR^{-/low}$ cells were able to suppress effectively both the proliferation of T cells in the presence of anti-CD3/anti-CD28 mAb. These results suggest that $CD14^+HLA\text{-}DR^{-/low}$ cells in ESCC patients contribute to the immune suppressive status. To further investigate the mechanism of this immune suppression, we examined the expression of co-stimulatory molecules which play important role in the regulation of T cell responses. Our data indicated that $CD14^+HLA\text{-}DR^{-/low}$ cells from patients with ESCC express a higher level of PD-L1 compared with those from healthy donors, whereas other co-stimulatory molecules such as CD80, CD86, B7-H3, and B7-H4 were not found to be significantly different. Similarly, PD-L1 highly expressed $CD14^+HLA\text{-}DR^{-/low}$ cells were also detected in squamous cell carcinoma of the head and neck [22].

As an important co-inhibitor molecule, programmed death ligand 1 (PD-L1), is able to suppress T cell response effectively. Recently, in clinical trials, antibodies against PD-1 and PD-L1 were used to block PD-1/PD-L1 pathway for the treatment of advanced cancers (including non-small cell lung cancer, melanoma, and renal cell cancer) and yielded encouraging results [44, 45]. It suggests that PD1/PD-L1 pathway, as an important immune checkpoint, is of major importance in tumor immune resistance. Blockage of PD1/PD-L1 pathway may be a novel immunotherapy for cancer. To confirm whether PD-L1 contributed to $CD14^+HLA\text{-}DR^{-/low}$ cell-mediated suppressive activity, we examined the effect of PD-L1 by using a blocking antibody against PD-L1 in the suppressing experiment. As expected, we found blocking PD-L1 could effectively reverse the suppressive activity of $CD14^+HLA\text{-}DR^{-/low}$ cells. Thus, the preferential expression of PD-L1 could contribute to immunosuppressive properties of $CD14^+HLA\text{-}DR^{-/low}$ cells in ESCC patients.

To sum up, our data suggest that in patients with ESCC, $CD14^+HLA\text{-}DR^{-/low}$ cells act as potent immunosuppressive cells, particularly contributing to the tumor immune escape from the host immune system. Furthermore, the frequency of circulating $CD14^+HLA\text{-}DR^{-/low}$ cells might provide a novel but not independent prognostic indicator of ESCC. Additionally, PD1/PD-L1 pathway may be an immune checkpoint of tumor immune resistance mediated by $CD14^+HLA\text{-}DR^{-/low}$ cells.

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Conflicts of interest None

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