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Significance of myeloid-derived suppressor cells (MDSCs) like CD14+B7-H4 cells frequency in blood and tumor microcirculation of lung cancer patients

Značaj učestalosti populacije CD14$^+$B7-H4$^+$ ćelija koje odgovaraju mijeloidnim supresivnim ćelijama (MDSC) u krvi i tumorskoj mikrocirkulaciji bolesnika sa karcinomom pluća

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Abstract

Background/Aim. Myeloid-derived suppressor cells (MDSCs) suppress immune responses *via* a series of inhibitory mechanisms, which ultimately could lead to tumor growth. B7-H4 expression is significantly associated with poor outcome and promotion of tumor cell proliferation, invasion and migration in patients with various cancers. Data concerning B7-H4 expression in lung cancers (LC), either on tumor or immunological cells, are still sporadic. The aim was to estimate and correlate the number of CD14$^+$B7-H4$^+$MDSCs in blood and lung tumor microcirculation with clinical stage, histology type of tumor, tumor node metastasis (TNM) stadium, nodal status and disease outspread. **Methods.** The study included 44 lung cancer patients (III and IV clinical stage) and 30 healthy controls. CD14$^+$B7-H4$^+$ MDSC number was estimated by flow cytometry in blood and tumor microcirculation samples of each patient. **Results.** CD14$^+$B7-H4$^+$ MDSCs number was significantly higher in patients' samples compared to controls. CD14$^+$B7-H4$^+$ MDSC number was significantly increased in tumor compared to blood sample of the same patient. Clinical stage III patients had the increased number of the CD14$^+$B7-H4$^+$ MDSC compared to stage IV, in both types of samples. According to histology, small cell lung cancer (SCLC) patients had the highest average CD14$^+$B7-H4$^+$ MDSCs number, significantly increased compared to patients with squamous and large cell LC histology type. Tumor size was directly associated with the number of the CD14$^+$B7-H4$^+$ MDSC, both in blood and tumor samples. Furthermore, nodal involvement was associated with the gradual increase of the CD14$^+$B7-H4$^+$ MDSC number, being the highest in the N3 group, again both in blood and tumor samples. Finally, we detected higher CD14$^+$B7-H4$^+$ MDSCs number in the samples of patients without metastases. **Conclusion.** CD14$^+$B7-H4$^+$ MDSCs number in LC patients is significantly associated with tumor histology type, lymph node involvement, disease extent degree and tumor size. Concerning their large number in LC tumor microenvironment together with immunosuppressive capacities, CD14$^+$B7-H4$^+$ MDSCs could represent important tumor promoting factor in LC pathophysiology.

Key words:
lung neoplasms; myeloid-derived suppressor cells; immunologic factors; neoplasm metastasis; flow cytometry; histology.

Apstrakt

Uvod/Cilj. Mijeloidne supresorske ćelije [*myeolid derived suppressor cells* (MDSCs)] negativno regulišu imunski odgovor nizom inhibitornih mehanizama koji konačno omogućavaju rast tumora. Ispoljavanje B7-H4 je značajno povezano sa lošim ishodom, kao i promocijom proliferacije, invazije i migracije ćelija tumora kod bolesnika sa različitim tipovima

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karcinoma. Podaci koji prikazuju ispoljavanje B7-H4 u tumorima pluća, na tumorskim ili imunskim ćelijama, su i dalje retki. Cilj rada bio je utvrditi i korelirati zastupljenost $CD14^+B7\text{-}H4^+$ ćelija sličnih MDSCs ($CD14^+B7\text{-}H4^+$ MDSCs) u krvi i mikrocirkulaciji tumora pluća sa kliničkim stadijumom, histološkim tipom tumora, tumor nodus metastaza (TNM) stadijumom, nodalnim statusom i raširenošću bolesti. **Metode.** U studiju je bilo uključeno 44 bolesnika sa karcinomom pluća (III i IV klinički stadijum) i 30 zdravih osoba. Zastupljenost (%) $CD14^+B7\text{-}H4^+$ MDSCs je bila utvrđena protočnom citometrijom u krvi i mikrocirkulaciji tumora svakog bolesnika. **Rezultati.** Zastupljenost $CD14^+B7\text{-}H4^+$ MDSCs bila je značajno veća u uzorcima bolesnika u odnosu na kontrole. $CD14^+B7\text{-}H4^+$ MDSCs u uzorku tumora su bile značajno brojnije u odnosu na uzorak krvi istog bolesnika. Bolesnici III kliničkog stadijuma imali su povećane vrednosti $CD14^+B7\text{-}H4^+$ MDSCs u odnosu na one u IV stadijumu, u obe vrste uzoraka. Prema histološkom tipu, bolesnici sa sitnoćelijskim karcinomom pluća imali su najveće vrednosti $CD14^+B7\text{-}H4^+$ MDSCs, značajno povećane u odnosu na bolesnike sa skvamoznim ili giganto-ćelijskim tipom tumora. Veličina tumora bila je direktno udružena sa brojem $CD14^+B7\text{-}H4^+$ MDSCs, u obe vrste uzoraka. Zahvaćenost limfnih žlezda bila je udružena sa postepenim povećanjem vrednosti $CD14^+B7\text{-}H4^+$ MDSCs, sa najvećim vrednostima u N3 grupi, u obe vrste uzoraka. Na kraju, detektovali smo veće vrednosti $CD14^+B7\text{-}H4^+$MDSCs u uzorcima bolesnika bez metastaza. **Zaključak.** Vrednosti $CD14^+B7\text{-}H4^+$ MDSCs kod bolesnika sa karcinomom pluća značajno su povezane sa histološkim tipom tumora, zahvatanjem limfnih čvorova, stepenom raširenosti bolesti i veličinom tumora. S obzirom na visoke vrednosti u mikrookruženju tumora pluća zajedno sa njihovim imunosupresivnim kapacitetima, $CD14^+B7\text{-}H4^+$ MDSCs mogu predstavljati važan promovišući faktor u patofiziologiji karcinoma pluća.

Ključne reči:
pluća, neoplazme; ćelije supresori; imunološki faktori; neoplazme, metastaze; citometrija, protočna; histologija.

Introduction

Lung cancer (LC) is the most common carcinoma in men while in women it is the fourth most common malignancy, and the second by lethal outcome [1]. At the moment of diagnosis, more than 50% of patients are in a locally advanced stage or have distant metastases, with poor five-year survival even in patients with localized disease.

Focus of contemporary immunotherapy is tumor-mediated immune suppression and modulation of tumor specific T lymphocyte activity by acting on checkpoint immune inhibitors. Beside programmed death (PD-1), PD-1 ligand and CTLA-4, new data describe other members of B7 accessory molecules that critically regulate activation or suppression of T lymphocytes [2]. Among them, B7-H4 (B7S1, B7x, Vtcn1) is a strong inhibitor of T cell activity [3]. Investigation of B7-H4 mRNA demonstrated broad presence in human non-lymphoid tissues. Expression of B7-H4 is documented in various solid malignant tumors, and its presence on tumor cells is associated with the increased rate of proliferation, metastasis and unfavorable outcome in patients with kidney cancer, oral and esophageal squamous carcinoma, gastric and LC [2]. Sica et al. [3] and Prasad et al. [4] were among first that demonstrated that B7-H4 expression is not restricted to tumor cells. They described that *in vitro* stimulation induced B7-H4 expression on population of T lymphocytes, majority of B lymphocytes and monocytes/macrophages. Macrophages isolated from ovarian cancer or from ascites of patients with ovarian cancer demonstrated significant expression of B7-H4 and potently inhibited *in vitro* T lymphocyte activation [5]. Furthermore, B7-H4 expression and suppressive capacity could be stimulated on macrophages after incubation with interleukin (IL)-10 and IL-6 [6]. Recently, these suppressive population of B7-H4 macrophages have been demonstrated in patients with glioma [7]. Li et al. [8] investigated possible mechanisms involved in generation of exhausted $CD8^+$ tumor infiltrating T lymphocytes in patients with hepatocellular carcinoma. They showed that the expression of B7-H4 on myeloid cells in tumor is in direct correlation with inhibition of $CD8^+$ T lymphocytes activity.

Myeloid-derived suppressive cells (MDSCs) represent significant force that supports tumor in survival, proliferation and metastasis. Experimental models strongly support significant role of MDSCs in immunosuppressive balance that favors survival and growth of lung tumors [9]. Inefficiency of recent trials with inhibitors of immune checkpoint in patients with non-small cell lung carcinoma (NSC-LC) is explained to be a consequence of significant immunosuppressive state caused by MDSCs activity [10]. Patients with NSC-LC demonstrate high numbers of both monocytic and polymorphonuclear MDSCs in peripheral circulation and tumor tissue [11]. MDSCs number directly correlate with inflammatory cytokines concentration, both locally and systemically, and the majority of MDSCs express PD-L1 as phenotypic marker of high immunosuppressive capacity. Yamauchi et al. [11] demonstrated that frequency of MDSCs is significantly correlated with the disease course and outcome in their cohort of NSC-LC patients. PD-L1 is not the only immunosuppressive molecule detected on MDSCs in LC patients. Zhang et al. [12] identified two populations of MDSCs in tumor tissue of NSC-LC patients according to the presence of inhibitory B7-H3 molecule. Monocyte like MDSCs population ($HLA\text{-}DR^{-/low}$, $CD14^+$MDSC) that expresses B7-H3 is highly immunosuppressive, produces significant amounts of IL-10 and tumor necrosis factor (TNF)-α and the increase of their number is significantly associated with short disease free interval. Considering all these data, in our study we wanted to investigate MDSCs population that express B7-H4 in LC patients, another inhibitory molecule, and to analyze association of their value with pathological and clinical parameters.

Methods

Patients

The study enrolled 44 patients with diagnosed LC (33 males 11 females, 62 ± 8 years) and 30 healthy controls (22 males, 8 females, 57 ± 14 years). Patients were diagnosed and treated at the Clinic for Pulmonology, Military Medical Academy in Belgrade, Serbia, in 18-month-long period. All necessary diagnostic procedures (laboratory, radiological, bronchoscopy and histological) were carried out at the Military Medical Academy in Belgrade, Serbia. All the patients as well as healthy controls signed the Informed consent for participation in the research. This study was approved by the Ethics Committee of the Military Medical Academy in Belgrade, Serbia (12-02/2015).

Samples

Blood samples were taken from the cubital vein upon admission to the hospital, while tumor microcirculation samples were taken by needle biopsy from available pathological tumor blood vessels in the course of diagnostic bronchoscopy. Samples were taken in vacutainer tubes with K_2EDTA and erythrocytes removed using the lysing buffer (NH_4Cl, EDTA, $KHCO_3$) for 10 min with constant mixing. The remaining nucleated cells were washed two times with the RPMI 1640 culture medium complemented with 5% of normal human serum, centrifuged, resuspended, enumerated (Beckman Coulter ACT differ blood counter) and concentration was corrected to final suspension of 1×10^6 cells/100 µL *per* test tube.

Cells immunophenotyping

Final cell suspensions were stained with coctail of monoclonal antibodies, as Stanojević et al. [13] did in the previous study. Multicolor analysis was performed with different combinations of CD15-FITC or PECy7, CD33-PE or PECy7, CD45-ECD or PECy5, HLA-DR PE/Cy5, CD14-FITC, CD16-PE, CD11b-PE, CD10-PECy7, CD3-FITC, CD19-FITC, CD56-FITC, B7-H4-PECy7 (Biolegend, USA). The flow cytometry was performed using a Beckman Coulter FC 500 flow cytometer with CXP analysis software. MDSCs subpopulation was identified from the initial $CD45^+$/Side Scatter cell population, which was negative for T, B and NK antigens. This triple negative population of every sample was further gated on a CD11b versus HLA-DR dot plot histograme, and MDSCs were analyzed as lineage triple negative ($CD3^-$, $CD19^-$, $CD56^-$), $CD45^+$, HLA-$DR^{-/low}$, $CD11b^+$ and $CD33^{low}$ population. After further classification of this population according to the expression of CD15 or CD14, $CD14^+$ MDSCs population was further investigated for B7-H4 expression. The value of MDSCs $CD14^+$ B7-$H4^+$ cell population was expressed as % of all $CD45^+$ analyzed cells, as we did in our previous work [13].

Statistical analysis

Data analysis was performed using the GraphPad Prism 5 software. Comparison between multiple groups was done with nonparametric Kruskal–Wallis test, while identification of differences was performed with Dunn`s multiple comparison test. Difference between average values of two investigated groups was analysed with Mann Whitney (MW) test, while statistical significance of serial samples (blood/tumor microcirculation) of patients was analysed with Wilcoxon (W) matched pairs test.

Results

Lung cancer patients had significantly higher number of the $CD14^+$ B7-$H4^+$MDSCs than controls

A statistically significant number of $CD14^+$B7-$H4^+$ MDSCs was detected in samples of peripheral blood of LC patients as well as in tumor microcirculation compared to peripheral blood samples of control patients ($p = 0.0417$, MW test). Generally, all patients (excluding 3 that had values as healthy controls) demonstrated significantly higher number of $CD14^+$B7-$H4^+$ MDSCs in tumor microcirculation samples compared to their blood values, either after comparison of average number ($p = 0.0002$, MW test) or when assessing particular tumor/blood samples of each patient ($p = 0.0000$, W test) (Table 1).

Clinical stage III patients had insignificantly increased the number of the $CD14^+B7$-$H4^+$ MDSCs compared to clinical stage IV patients

Comparison of patients between the III and the IV clinical stage demonstrated no significant difference, either in blood or tumor microcirculation samples (Table 1). Again, all patients, in both clinical stages had significantly more of the $CD14^+$B7-$H4^+$ MDSCs in their correspondive tumor microcirculation than blood samples (Table 2).

Patients with small cell LC histology demonstrated the highest average CD14+ B7-H4+MDSC values in tumor microcirculation samples

Stratification of patients in groups related to histological type of the tumor revealed differences in $CD14^+$B7-$H4^+$ MDSCs number according to different histology. Although all groups had more $CD14^+$B7-$H4^+$ MDSCs in their tumor compartment compared to blood, these differences reached statistical significance for small cell LC, adenocarcinoma and squamous LC, but not for patients with large cell LC (Table 2, W test). Furthermore, analysis of the average $CD14^+$B7-$H4^+$ MDSCs number in tumor/blood compartment demonstrated significant increase only in tumor microcirculation of small cell LC and squamous cell LC groups (Table 2, MW test). Comparison between groups of patients with different lung cancer histology type in blood samples revealed no statistical significance, although, again, almost all patients had values higher than normal controls. But, analysis of tumor microcirculation

samples demonstrated that patients with small cell LC had the highest average $CD14^+B7\text{-}H4^+$ MDSC number, significantly increased compared to patients with squamous and large cell LC histology (Table 3, MW test).

Smallest tumors are significantly associated with the lowest $CD14^+B7\text{-}H4^+$ MDSC number

Blood samples of the LC patients with the smallest tumors (T1) demonstrated significantly low $CD14^+B7\text{-}H4^+$ MDSCs value, close to the number of healthy controls (Table 1). The increment of the tumor was significantly associated with the increase of $CD14^+B7\text{-}H4^+$ MDSCs number, compared to this group (T2 > T1, T3 > T1, T4 > T1, Table 3, blood, MW test). Interestingly, patients with T3 tumors had significantly increased number of the $CD14^+B7\text{-}H4^+$ MDSCs compared to those with the largest tumors (T3 > T4, Table 3, MW test). Analysis of tumor microcirculation compartment demonstrated almost the same relations as in blood, meaning that the patients with

Table 1

Presentation of $CD14^+B7\text{-}H4^+$ MDSCs in blood and tumor microcirculation samples of lung cancer (LC) patients according to clinical status and tumor characteristics

Parameter	MDSCs (% of total $CD45^+$ cells), mean ± SD	
	blood	tumor microcirculation
Clinical stage		
III (n = 27)	5.96 ±5.42	26.93 ± 22.03
IV (n = 17)	5.38 ± 4.72	23.94 ± 21.28
Histology type of LC		
Ad NSCLC (n = 13)	4.40 ± 4.88	24.10 ± 30.05
Sq NSCLC (n = 11)	7.50 ± 5.63	26.83 ± 20.31
Lc NSCLC (n = 10)	6.00 ± 5.15	9.40 ± 9.86
SCLC (n = 10)	6.10 ± 5.78	41.56 ± 9.45
Tumor size		
T1 (n = 11)	1.46 ± 1.37	6.82 ± 7.14
T2 (n = 13)	7.07 ± 5.44	30.43 ± 23.20
T3 (n = 13)	9.29 ± 6.15	28.57 ± 22.62
T4 (n = 7)	4.38 ± 2.45	27.00 ± 16.83
Nodal status		
N0 (n = 11)	3.00 ± 2.68	7.55 ± 7.83
N1 (n = 10)	4.50 ± 2.64	14.50 ± 13.79
N2 (n = 13)	7.15 ± 5.98	29.54 ± 21.62
N3 (n = 10)	14.67 ± 4.39	33.44 ± 16.27
Metastases		
M0 (n = 27)	27	20.43 ± 11.63
M1 (n = 17)	17	17.50 ± 3.97

***Note*: MDSCs value in blood samples of the control group (n = 30) was 1.04 ± 0.32. MDSCs – myeloid-derived suppressive cells; NSCLC – non small cell lung carcinoma; Ad – adenocarcinoma; Sq – squamose; Lc – large cell; SCLC– small cell lung carcinoma.**

Table 2

Comparison of $CD14^+B7\text{-}H4^+$ MDSCs presentation between blood and tumor microcirculation compartments in lung cancer (LC) patients according to clinical status and timor characteristics

Parameter	Compartment		Statistical tests	
	blood	tumor microcirculation	Mann Whitney	Wilcoxon
Clinical stage	III	III	$p = 0.0027$	$p = 0.0000$
	IV	IV	$p = 0.0122$	$p = 0.0007$
Histology type	SCLC	SCLC	$p = 0.0003$	$p = 0.0088$
	Ad NSCL	Ad NSCLC	ns	$p = 0.0213$
	Sq NSCLC	Sq NSCLC	$p = 0.0025$	$p = 0.0005$
	Lc NSCLC	LC NSCLC	ns	ns
Tumor size	T1	T1	$p = 0.0238$	$p = 0.0089$
	T2	T2	$p = 0.0011$	$p = 0.0038$
	T3	T3	$p = 0.0049$	$p = 0.0037$
	T4	T4	$p = 0.0021$	$p = 0.0156$
Nodal status	N0	N0	$p = 0.0362$	ns
	N1	N1	$p = 0.0141$	$p = 0.0112$
	N2	N2	$p = 0.0037$	$p = 0.0126$
	N3	N3	$p = 0.0091$	$p = 0.0017$
Metastases	M0	M0	ns	$p = 0.0028$
	M1	M1	$p = 0.0000$	$p = 0.0025$

For abbreviations see under Table 1.

the smallest tumors had significantly less $CD14^+B7\text{-}H4^+$ MDSCs compared to all other groups (T2 > T1, T3 > T1, T4 > T1, Table 3, tumor microcirculation, MW test).

N3 nodal status was associated with the highest $CD14^+B7\text{-}H4^+$ MDSCs values

Patients without nodal involvement demonstrated the lowest number of $CD14^+B7\text{-}H4^+$ MDSCs both in the blood and tumor microcirculation compartment. Furthermore, nodal involvement was associated with the gradual increase of the $CD14^+B7\text{-}H4^+$ MDSCs number, being the highest in the N3 group (Table 1). There was a significant increase in tumor microcirculation number of the $CD14^+B7\text{-}H4^+$ MDSCs compared to that in the blood, either when analysed as a group (Table 2, MW test) or in particular tumor/blood samples of each patient (Table 2, W test). Analysis among groups with different nodal involvement demonstrated that N3 group had significantly more $CD14^+B7\text{-}H4^+$ MDSCs than any other group, either in blood or tumor (N3 > N0, N3 > N1, N3 > N2) (Table 3). Additionally, the N2 group also demonstrated a significant increase of the $CD14^+B7\text{-}H4^+$ MDSCs number compared to the N0 group, both in blood and tumor microcirculation (N2 > N0) (Table 3).

Higher $CD14^+B7\text{-}H4^+$ MDSCs values detected in the group M0

Although we detected higher average $CD14^+B7\text{-}H4^+$ MDSCs value in the M0 group (Table 1), a comparison of patients between the M0 and the M1 group demonstrated no significant difference, either in blood or tumor microcirculation samples (Table 3). Comparison of blood/tumor average $CD14^+B7\text{-}H4^+$ MDSCs values demonstrated only significant differences in the M1 group (Table 2 MW test). Analysis of particular tumor/blood samples of each patient demonstrated significant increase of the $CD14^+B7\text{-}H4^+$ MDSCs number in tumor microcirculation (Table 2, W test).

Discussion

Expression of immunosuppressive B7-H4 molecule was widely demonstrated in samples of tumor tissue from patients with gynecological malignancies (ovary, uterus), as well as in patients suffering from colon and pancreas carcinoma [14, 15]. In physiological condition, B7-H4 is absent from the surface of normal cells [16]. Beside malignant cells, B7-H4 is extensively detected on the surface of tumor infiltrating macrophages with up to 2/3 of the tumor ascites

Table 3

Statistical analysis of difference between groups of patients according to clinical stage and tumor characteristics (Mann Whitney test)

Group	vs.	Group	Blood	Tumor
Histology type				
SCLC	/	Ad NSCLC	ns	ns
SCLC	/	Sq NSCLC	ns	0.0180
SCLC	/	LC NSCLC	ns	0.0010
Ad NSCLC	/	Sq NSCLC	ns	ns
Ad NSCLC	/	LC NSCLC	ns	ns
Sq NSCLC	/	LC NS-LC	ns	ns
Tumor size				
T1	/	T2	0.0029	0.0036
T1	/	T3	0.0004	0.0056
T1	/	T4	0.0040	0.0023
T2	/	T3	ns	ns
T2	/	T4	ns	ns
T3	/	T4	0.0442	ns
Nodal status				
N0	/	N1	ns	ns
N0	/	N2	0.0452	0.0042
N0	/	N3	0.0000	0.0002
N1	/	N2	ns	ns
N1	/	N3	0.0000	0.0137
N2	/	N3	0.0044	ns
Metastases				
M0	/	M1	ns	ns
Clinical stage				
III	/	IV	ns	ns

ns – no significant.
For other abbreviations see under Table 1.

$CD14^+$ macrophages being also B7-$H4^+$ [17, 18].

More than 10 years ago Ilona Kreyczek group demonstrated the importance of B7-$H4^+$ macrophages in human ovarian carcinoma [5, 6]. They investigated $CD14^+$ monocytes, B7-$H4^-$ and B7-$H4^+$ macrophages as well as regulatory T lymphocytes isolated from fresh tumor specimens, tumor induced ascites and blood of 103 patients with ovarian carcinoma. Although tumor cells also expressed B7-H4 intracellularly, only tumor infiltrating macrophages demonstrated surface B7-$H4^+$ expression. This B7-$H4^+$ expression was highly inducible, since it was possible to transform peripheral blood monocytes with tumor ascites or addition of IL-6 and IL-10. Interestingly, IL-4 and granulocyte-macrophage colony-stimaluting factors (GM-CSF) negatively regulate *ex vivo* and *in vitro* B7-$H4^+$ expression on macrophages. Authors initially concluded that the change of local factors concentration, with high IL-6 and IL-10, and low IL-4 and GM-CSF resulted in the transformation of tumor-associated macrophages toward B7-$H4^+$ cells. More importantly, Kreyczek et al. [18] further demonstrated that B7-$H4^+$ macrophages suppressed *in vitro* activity of T lymphocytes specific for HER2/Neu antigen, in a way independent of B7H1 mechanism, arginase or iNOS activity. Considering a high number of B7-$H4^+$ macrophages detected in tumor-associated ascites, which largely outnumbered regulatory T lymphocytes (30% vs. 5%), authors concluded that these cells could be principal immunosuppressive force, resulting in tumor promotion.

Matsunaga et al. [19] investigated association of blood and tumor $CD14^+B7H1^+$ or $CD14^+$B7-$H4^+$ cells with clinical and tumor characteristics in the patients with gastric cancer. Firstly, they demonstrated that level of $B7H1^+$ or B7-$H4^+$ expression is significantly increased on monocytes from gastric cancer patients, compared to healthy controls. They also found that tumor isolated monocytes expressed significantly more $B7H1^+$ or B7-$H4^+$ compared to blood monocytes from the same patient, and that the expression of these suppressive molecules is directly correlated. Contrary to our study, the expression of B7-$H4^+$ directly followed HLA-DR expression level on $CD14^+$ cells. Differences come from different gating strategies, since our goal was to investigate MDSCs which are HLA-$DR^{-/low}$ by definition. Anyway, these authors demonstrated significant immunosuppressive capacity of $CD14^+$ B7-$H4^+$ cells *in vitro* (reduction of interferon (IFN)-γ secretion by T lymphocytes), and also demonstrated that surgical removal of tumor resulted in decreased B7-$H4^+$ on circulating $CD14^+$ cells. They demonstrated differences in blood and tumor $CD14^+B7H1^+$ or $CD14^+$B7-$H4^+$ number according to histopathology type of gastric cancer, invasion depth, tumor size, node involvement, clinical stage and a level of lymphovascular invasion. Their data is in accordance with the data obtained by our study, indicating that the tumor size and the degree of the disease spread is directly associated with the number of suppressive $CD14^+$B7-$H4^+$ number. Interestingly, their patients in the earlier clinical stage also demonstrated insignificantly higher $CD14^+$B7-$H4^+$ number compared to later stages.

Data concerning MDSCs role in patients with lung cancer are still limited, especially investigations of B7-$H4^+$ MDSCs.

Investigation of prognostic B7-H4 value in patients with NSC-LC conducted as a meta-study by Tan and Shen [20] indicated significant association of B7-H4 overexpression with tumor size, node involvement and the presence of metastasis, but without the impact of tumor histology and other epidemiological factors. They concluded that B7-H4 expression is a negative prognostic factor for NSC-LC patients. Unfortunately, their study included one big imprecision, since in their selection criteria (criteria Nº 2) they included studies with B7-H4 expression detected with any method, which implies that they did not differentiate expression on malignant tissue or tissue infiltrating leukocytes.

Chen et al. [21] investigated $CD14^+$HLA-$DR^{-/low}$ MDSCs frequency in blood samples of almost 80 patients with squamous type of LC (Sq NSC-LC). They also demonstrated that LC patients had significanlty increased number of these cells compared to healthy controls, and that MDSCs number gradually rise in those patients with higher TNM stage. In favor of immunosuppressive MDSCs impact, they showed significantly reduced number of blood $CD4^+$ T and $CD8^+$ T lymphocytes in patient samples, as well as the impairment of T lymphocyte cytokine secretion *in vitro*.

Dendritic cells (DC) isolated from resected tumor tissue of NSC-LC patients express other immunosupressive molecules, also from B7 family, as B7H3 [22]. Those DC demonstrated severely impaired costimulatory activity towards autologous T lymphocytes, produced significantly more IL-10 and less IL-12 than controls.

Huang et al. [23] demonstrated that circulating $CD14^+$HLA-$DR^{-/low}$ MDSC are modulators of antitumor immune response and were associated with tumor metastasis and impaired response to treatment in NSCLC patients [23]. They evaluated 89 patients with advanced NSCLC. The ratio of the $CD14^+$HLA-$DR^{-/low}$ MDSCs, as a percent of total $CD14^+$ cells, was significantly higher in NSCLC patients compared to healthy controls, and it was proportional to clinical stage. Monocytic MDSCs also significantly negatively correlated with median progression-free survival ($p < 0.01$). Both the absolute number and percentage of $CD14^+$HLA-$DR^{-/low}$ cells were increased in NSCLC patients with metastasis, confirming their role in the disease progression. Huang et al. [23] also demonstrated that the role of $CD14^+$HLA-$DR^{-/low}$ cells in inhibition of T cell function of NSCLC patients was mediated by a functional NADPH oxidase, as shown by the expression of the oxidase component gp91phox and reactive oxygen species (ROS) production by these monocytic MDSCs.

Ex vivo study showed that circulating tumor cell lines from SCLC patients induced transformation of peripheral blood mononuclear cells (PBMNC) toward diferentiaton of monocytes in $CD14^+$ $CD163^{low}$ $CD68^+$ B7-$H4^+$ tumor associated macrophages [24]. Feng et al. [25] underlined the significance of MDSCs in patients with adenocarcinoma LC (Ad NSC-LC) positive for epidermal growth for receptor

(EGFR) mutation. They also showed that MDSCs number ($S100A^+CD68^+$) was increased in patient blood samples compared to healthy controls. Patients with a poor therapy response as well as patients with short progression free interval had increased MDSCs number compared to others. Again, as in our study, the number of MDSCs was much higher in tumor samples compared to matched blood ones. A recent study of blood MDSCs subpopulations in the NSC-LC patients demonstrated that they were increased in number compared to healthy controls, but also showed the increased number in a subgroup of COPD as second controls [11]. The same study showed that MDSCs were more frequent in resected tumor tissue than in blood samples of these NSC-LC patients. Data from that study demonstrated fine differences between LC with different histology. Patients with squamous type LC showed the increased number of granylocyte like MDSCs compared to the adenocarcinoma group, but without differences in monocyte like MDSCs. This was in concordance with our data, since we showed no significant differences in $CD14^+$ MDSCs frequency between NSC-LC patients, but only between small cell LC group versus others. On the other hand, Yamauchi et al. [11] showed that adenocarcinoma LC group had significantly more $CD14^+B7H1^+$ MDSCs and $CD15^+B7H1^+$ MDSCs compared to squamous cell LC patients. In our previous study we have demonstrated that different histology type of LC is significantly associated with particular cytokine profile, either in blood or tumor microcirculation samples [26].

In the study with limited number of NSC-LC patients, Pogoda et al. [27] demonstrated that $CD14^+HLA\text{-}DR^{-/low}$ MDSCs are not the only population that takes part in tumor induced immunosuppression. Beyond MDSCs, they showed that $CD14^+HLA\text{-}DR^+$ monocyte population secreted significant amounts of IL-10 in lymph node samples, and IL-1β and TNF in peripheral blood, lymph nodes and tumor tissue. Heuvers et al. [28] investigated MDSCs frequency in blood specimens of 185 NSC-LC patients demonstrating the increased number of MDSCs, especially granulocyte like, in LC patients compared to healthy controls. They also showed that the suppression capacity of MDSCs is significantly associated with arginase -1 activity.

The majority of published papers reflect investigations in NSC-LC patient population. In a study that involved 42 SC-LC patients, Tian et al. [29] demonstrated that the absolute number and frequency of blood $CD14^+HLA\text{-}DR^{-/low}$ MDSCs were significantly increased in SCLC patients compared with those in controls and that the MDSCs frequency correlated with tumor stage, serum lactate dehydrogenase (LDH) value and shorter overall survival. SCLC patients from our study demonstrated the highest average number of $CD14^+HLA\text{-}DR^{-/low}$ MDSCs, but in tumor microcirculation samples.

Conclusion

We demonstrated a significant association between the number of $CD14^+B7\text{-}H4^+$ MDSCs and the tumor size and lymph node involvement. We also showed that LCs of different histology dramatically differ in their capacity to induce $CD14^+B7\text{-}H4^+$ MDSCs number, which could be interpreted as different immunosuppression potential. We found that tumor microcirculation samples are easily available for analysis and more important than blood samples, offering more sensitive and informative data, more precisely reflecting the local balance between tumor and immune response.

Conflict of interest

Authors declare no conflict of interest.

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