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ОРИГИНАЛЬНОЕ ИССЛЕДОВАНИЕ
ORIGINAL RESEARCH

Tumor hybrid cells in non-small cell lung cancer: population structure and contribution to prognosis

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Abstract. *Relevance*. Non-small cell lung cancer (NSCLC) is the leading cause of cancer-related mortality worldwide due to the high recurrence and metastasis rates. It is generally accepted that metastases and recurrences are formed by tumor cells with a highly invasive, stem and chemoresistant phenotype. Tumor hybrid cells (THCs) formed by the fusion of tumor cells with a wide range of normal cells: macrophages, fibroblasts, mesenchymal stem cells, etc. are considered to be potential metastasis and recurrence-initiating cells. However, the phenotypic diversity of THCs, and their association with disease progression remain poorly understood. *The aim* of the study was to characterize the population composition of THCs in NSCLC and its association with clinicopathological characteristics, metastasis and recurrence. *Materials and Methods*. A total of 50 patients with NSCLC were included. Fresh frozen samples of tumor tissue obtained during resection and morphologically verified were used to analyze types and number of THCs. THCs were analyzed by flow cytometry in primary tumors using markers for tumor cells, cancer stem cells, leukocytes, macrophages and fibroblasts. *Results and Discussion.* THCs were detected in all NSCLC patients. Most THCs demonstrated leukocyte, macrophage and stem characteristics. The number and frequency of THCs depended on neoadjuvant chemotherapy. THCs with leukocyte and stem cell markers (pan-CK$^+$CD45$^+$CD44$^+$CD73$^+$) were associated with locoregional recurrence, whereas THCs with macrophage and stem cell markers (EpCAM$^+$CD45$^+$CD44$^+$CD73$^+$CD163$^+$) — with distant metastases. *Conclusion*. This study is the first to comprehensively describe the population composition of THCs in NSCLC, their association with clinicopathological characteristics, neoadjuvant chemotherapy and disease prognosis. Detection of prognostically relevant THCs could be an effective approach for predicting the risk of metastasis and recurrence of NSCLC and the basis for the development of therapy focused on the prevention of cancer progression.

Keywords: tumor fusion, tumor hybrid cells, non-small cell lung cancer, flow cytometry, metastasis, recurrence

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Introduction

Lung cancer claims over a million lives around the world every year and is the leading cause of cancer-related mortality [1]. Non-small cell lung cancer (NSCLC) accounts for 85 % of all lung cancer cases [2]. The relative 5-year survival rate for NSCLC does not exceed 35 % [3]. Among patients without signs of tumor spread the value reached 65 % [3]. While among patients with lymph nodes or distant organ metastases the value was 37 % and 9 %, respectively. Thus, predicting and preventing the risk of NSCLC progression is an urgent task for modern oncology.

The main players in metastasis are considered to be abnormally motile cells of the primary tumor that have undergone epithelial-mesenchymal transition [4, 5, 6]. Deprived of intercellular contacts, such cells are able to penetrate into surrounding tissues and blood and lymphatic vessels, thereby ensuring spreading throughout the body [5]. Considering that most metastatic "seeds" die while circulating in the bloodstream, and their successful reaching the distal organ does not guarantee further development of metastases, it becomes obvious that true metastasis-initiating cells must have a certain phenotype that allows them to successfully overcome both mechanical stress and escape from the immunological surveillance [7].

Recurrences are most likely caused by populations of tumor cells and cancer stem cells that are capable of self-renewal and regeneration and are resistant to chemotherapy and radiation therapy [8]. However, consensus on the classification criteria, origin and clinical significance of recurrence-initiating cells has not been reached.

In recent years, there has been increasing evidence that metastases and recurrences can be formed by tumor hybrid cells (THCs) [9–11]. The phenomenon of hybrid cells is widely known in normal physiological processes, for example, in wound regeneration and healing, and osteoclastogenesis. However, these cells are also found in pathological conditions, for example, in cancer [12, 13]. Tumor cells are capable of merging with both themselves and various immune cells: leukocytes (macrophages, dendritic cells, and lymphocytes), fibroblasts, mesenchymal stem, and other cells [14]. THCs acquire new properties that are not typical for parental cells, such as increased proliferation and migration, drug resistance, decreased apoptosis, and evasion of immune surveillance

[15–17]. THCs are found in a variety of malignancies and are associated with poor prognosis [18].

However, the phenotypic diversity of THCs remains poorly understood. In addition, most of the data have been obtained *in vitro* models and are valid for THCs of macrophage origin. In this regard, this study was aimed to characterize the population composition of THCs in NSCLC and its association with clinicopathological characteristics, metastasis and recurrence.

Materials and methods

The study included 50 patients with NSCLC treated at the Cancer Research Institute of the Tomsk National Research Medical Center (Table 1). Fresh frozen samples of tumor tissue obtained during resection and morphologically verified were used to analyze types and number of THCs.

All patients gave voluntary informed consent to participate in the study and personal data processing according with the World Medical Association Declaration of Helsinki (WMA Declaration of Helsinki — Ethical Principles for Medical Research Involving Human Subjects, 2013).

Preparation of cell suspension

Cell suspension was prepared from fresh frozen tumor samples. Tumor material was washed twice with phosphate-buffered saline (PBS) and placed in Dulbecco's Modified Eagle Medium (DMEM), serum-free culture medium, in which the tissue was minced with a scalpel into 1–2 mm fragments. Next, tumor fragments along with the medium were transferred into C-Tubes (Miltenyi Biotec, Germany) and mechanically homogenized using a gentleMACS Octo Dissociator device (Miltenyi Biotec, Germany). After homogenization, the resulting suspension was passed through a 70 μm cell filter (NEST Biotechnology, China).

The filtered cell suspension was centrifuged for 3 minutes at 300 rcf, the supernatant was removed, and the sediment was dissolved in PBS immediately before staining with an antibody cocktail.

Table 1

Clinicopathological characteristics of non-small cell lung cancer patients

Characteristics		N (%)
Age	62.5 ± 9.3	50 (100)
Sex	Male	39 (78)
	Female	11 (22)
Tumor size	T1	7 (14)
	T2	20 (40)
	T3	16 (32)
	T4	7 (14)
Lymphogenous metastasis	N0	23 (46)
	N1	14 (28)
	N2	13 (26)
Distant metastasis	Yes	19 (38)
	No	31 (62)
Stage	1	10 (20)
	2	12 (24)
	3	26 (52)
	4	2 (4)
Grade	G1	5 (10)
	G2	36 (72)
	G3	9 (18)
Locoregional recurrence	Yes	38 (76)
	No	12 (24)
Histologic type	Adenocarcinoma	25 (50)
	Squamous cell carcinoma	25 (50)
Neoadjuvant chemotherapy	Yes	20 (40)
	No	30 (60)
Smoking	Yes	40 (80)
	No	10 (20)

Flow cytometry

Markers of parental cells which were used to determine the number and types of THCs are presented in Table 2.

Table 2

Markers and antibodies used to identify tumor hybrid cells

Marker (manufacturer)	Dye	Antibody clone	Description
Live-or-Die (Biotium, Turkey)	405/545	–	Viability marker
CD45 (Beckman Coulter, USA)	APC-Alexa Fluor 700	J33	Leukocyte common antigen [19]
Pan-cytokeratin (pan-CK, Miltenyi Biotec, Germany)	FITC	REA831	Tumor cell marker [20]
CD326 (Elabscience, China)	Elab Fluor Violet 450	9C4	Tumor cell marker [21]442
S100A4 (BioLegend, USA)	PerCP Cy 5.5	NJ-4F3	Fibroblast marker [22]
FAP (eBioscience, USA)	Super Bright 600	F11–24	Fibroblast marker [23]
CD44 (BD, USA)	APC H7	C26	Cancer stem cell marker [24]
CD73 (Elabscience, China)	PE	AD2	Cancer stem cell marker [25]
CD68 (Elabscience, China)	Elab Fluor 647	Y1/82A	Macrophage marker [26]
CD163 (eBioscience, USA)	Super Bright 645	GHI/61	M2 macrophage marker [27]

The tumor cell suspension was stained with 85 µl solution contained of "Live-or-Die" dye and PBS, vortexed and incubated for 10 minutes. Then, monoclonal antibodies against CD45, CD163, FAP, CD44, CD73, and EPCAM were added and incubated for 15 minutes in dark. The cell suspension was then washed twice to remove unbound antibodies using a 0.5 % PBS+BSA (Bovine Serum Albumin) solution for 10 minutes at 2000 rpm. The precipitate was incubated with fixation buffer from the Flow Cytometry Fixation/ Permeabilization Kit (Biotium, USA) for 20 minutes. After washing twice 5 minutes at 2200 rpm with PBS and 2 % BSA, a permeabilization buffer from the Flow Cytometry Fixation/Permeabilization Kit (Biotium, USA) was added, which was mixed with antibodies to pan-cytokeratin, CD68 and S100A4. After 30 minutes of incubation in dark, the cell suspension was washed twice in 0.5 % PBS+BSA for 5 minutes at 2200 rpm. The supernatant was discarded, 100 µl of PBS was added to the cell pellet, mixed and analyzed on a CytoFLEX flow cytometer (Beckman Coulter, USA). To improve the accuracy of the results, take into account nonspecific antibody binding and adjust color compensation, FMO (Fluorescence Minus One) controls and unstained controls were used. The results were analyzed using the CytExpert program (Beckman Coulter, USA). The number of events attributed using selected markers to THCs was assessed. Data were extrapolated to 10000 cells. The gating strategy is shown in Fig. 1. Based on the forward (FSC) and side scatter (SSC) light parameters, an area with cells was selected and singlets were separated using a strategy for excluding doublets by area and height (FSC-A vs. FSC-H). Based on the fluorescence intensity of the "Live-or-Die" reagent, an area with living cells was selected, which were further gated by antibodies fluorescence intensity against markers of the target populations.

Statistical analysis

Statistical analysis was carried out using the SPSS Statistics v23 software (IBM, USA). The nonparametric Mann-Whitney test was used for two independent samples; data were presented as median (Me) and interquartile range (Q1–Q3). Association of overall, metastatic-free and recurrence-free survival with THCs was analyzed using the Kaplan-Meier method and the log-rank test. Association of clinicopathological characteristics and metastasis and recurrence frequency with THCs with were assessed using the Pearson's χ^2 test Differences were considered statistically significant at $p < 0.05$.

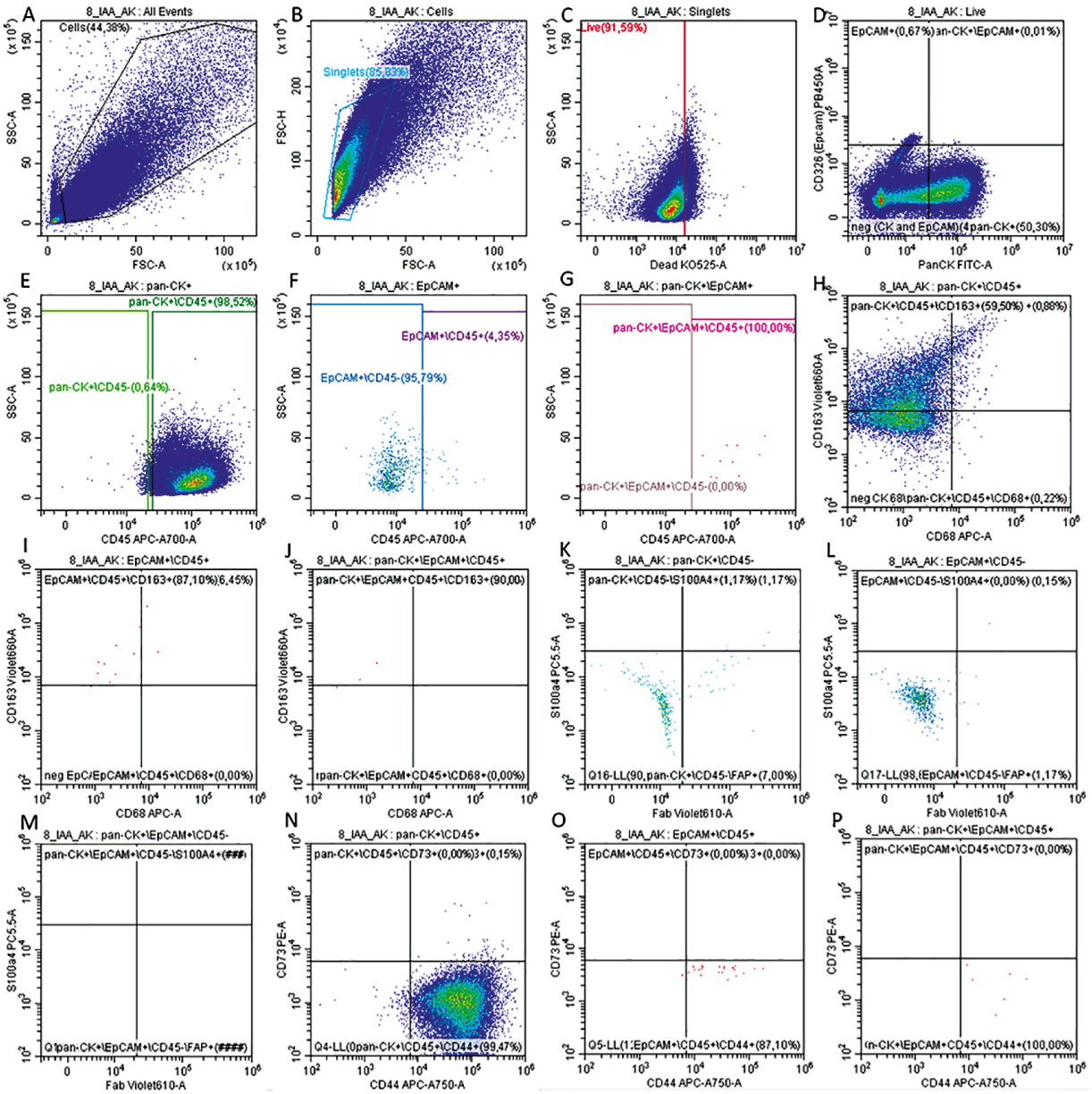

Figure 1. The flow cytometry algorithm for tumor hybrid cells identification

Note: **A** — Forward scatter (FSC) vs. side scatter (SSC) dot plot of cells; **B** — Singlets separation on the FSC-A/FSC-H histogram; **C**–Cell viability, as visualized by "Live-or-Dye" staining: positive cells are dead, negative cells are alive; **D** — Identification of pan-CK$^+$, EpCAM$^+$ and pan-CK$^+$EpCAM$^+$ cells; **E** — Identification of pan-CK$^+$CD45$^+$ and pan-CK$^+$CD45$^-$ cells; **F** — Identification of EpCAM$^+$CD45$^+$ and EpCAM$^+$CD45$^-$ cells; **G** — Identification of pan-CK$^+$EpCAM$^+$CD45$^+$ and pan-CK$^+$EpCAM$^+$CD45$^-$ cells; **H** — Identification of pan-CK$^+$CD45$^+$CD68$^+$, pan-CK$^+$CD45$^+$CD163$^+$ and pan-CK$^+$CD45$^+$CD68$^+$CD163$^+$ cells; **I**–Identification of EpCAM$^+$CD45$^+$CD68$^+$, EpCAM$^+$CD45$^+$CD163$^+$ and EpCAM$^+$CD45$^+$CD68$^+$CD163$^+$ cells; **J** — Identification of pan-CK$^+$EpCAM$^+$CD45$^+$CD68$^+$, pan-CK$^+$EpCAM$^+$CD45$^+$CD163$^+$ and pan-CK$^+$EpCAM$^+$CD45$^+$CD68$^+$CD163$^+$ cells; **K** — Identification of pan-CK$^+$CD45$^-$FAP$^+$, pan-CK$^+$CD45$^-$S100A4$^+$ and pan-CK$^+$CD45$^-$FAP$^+$S100A4$^+$ cells; **L**–Identification of EpCAM$^+$CD45$^-$FAP$^+$, EpCAM$^+$CD45$^-$S100A4$^+$ and EpCAM$^+$CD45$^-$FAP$^+$S100A4$^+$ cells; **M**–Identification of pan-CK$^+$EpCAM$^+$CD45$^-$FAP$^+$, pan-CK$^+$EpCAM$^+$CD45$^-$S100A4$^+$ and pan-CK$^+$EpCAM$^+$CD45$^-$FAP$^+$S100A4$^+$ cells; **N** — Identification of pan-CK$^+$CD45$^+$CD44$^+$, pan-CK$^+$CD45$^+$CD73$^+$ and pan-CK$^+$CD45$^+$CD44$^+$CD73$^+$ cells; **O** — Identification of EpCAM$^+$CD45$^+$CD44$^+$, EpCAM$^+$CD45$^+$CD73$^+$ and EpCAM$^+$CD45$^+$CD44$^+$CD73$^+$ cells; **P** — Identification of pan-CK$^+$EpCAM$^+$CD45$^+$CD44$^+$, pan-CK$^+$EpCAM$^+$CD45$^+$CD73$^+$ and pan-CK$^+$EpCAM$^+$CD45$^+$CD44$^+$CD73$^+$ cells.

Results and Discussion

THCs were detected in all NSCLC patients. In total, six populations and 84 subpopulations of THCs were identified. At the same time, 76 subpopulations were represented by at least one cell in one patient (Table 1, Supplement). Associations with clinicopathological characteristics, metastasis and recurrence were identified for 25 subpopulations of THCs (Table 3).

Table 3

Tumor hybrid cells populations and subpopulations associated with clinical features of NSCLC

THC populations	THC subpopulations	Abbreviations
THCs with leukocyte features	pan-CK$^+$CD45$^+$	TL1
	EpCAM$^+$CD45$^+$	TL2
	pan-CK$^+$EpCAM$^+$CD45$^+$	TL3
THCs with macrophage features	pan-CK$^+$CD45$^+$CD163$^+$	TM1
	EpCAM$^+$CD45$^+$CD163$^+$	TM2
	pan-CK$^+$EpCAM$^+$CD45$^+$CD68$^+$CD163$^+$	TM3
	pan-CK$^+$CD45$^+$CD68$^+$	TM4
THCs with fibroblast features	EpCAM$^+$CD45$^-$S100A4$^+$	TF1
	pan-CK$^+$CD45$^-$FAP$^+$S100A4$^+$	TF3
	pan-CK$^+$EpCAM$^+$CD45$^-$S100A4$^+$	TF4
THCs with stem and leukocyte features	pan-CK$^+$CD45$^+$CD44$^+$	TLS1
	EpCAM$^+$CD45$^+$CD44$^+$	TLS2
	pan-CK$^+$EpCAM$^+$CD45$^+$CD44$^+$	TLS3
	pan-CK$^+$CD45$^+$CD44$^+$CD73$^+$	TLS4
	pan-CK$^+$CD45$^+$CD73$^+$	TLS5
	EpCAM$^+$CD45$^+$CD44$^+$CD73$^+$	TLS6
THCs with stem and macrophage features	pan-CK$^+$CD45$^+$CD44$^+$CD163$^+$	TMS1
	pan-CK$^+$EpCAM$^+$CD45$^+$CD44$^+$CD68$^+$CD163$^+$	TMS2
	EpCAM$^+$CD45$^+$CD44$^+$CD73$^+$CD163$^+$	TMS3
	pan-CK$^+$CD45$^+$CD73$^+$CD68$^+$	TMS4
	pan-CK$^+$EpCAM$^+$CD45$^+$CD44$^+$CD73$^+$CD68$^+$CD163$^+$	TMS5
	pan-CK$^+$CD45$^+$CD44$^+$CD68$^+$	TMS6
THCs with stem and fibroblast features	pan-CK$^+$CD45$^-$CD44$^+$S100A4$^+$	TFS1
	pan-CK$^+$CD45$^-$CD44$^+$FAP$^+$S100A4$^+$	TFS2
	pan-CK$^+$CD45$^-$CD73$^+$FAP$^+$S100A4$^+$	TFS3

Tumor hybrid cells types, number and frequency in NSCLC

THCs with markers of leukocytes and macrophages, as well as THCs with stem, leukocyte and macrophage features were abundant (Table 4).

Table 4

Top 10 tumor hybrid cells subpopulations in NSCLC

THC subpopulations	Number of cells, Me (Q1-Q3)
TL1	1236 (453–2810)
TLS1	843 (340–2405)
TM1	285 (106–449)
TMS1	221 (87–427)
TF1	18 (1–38)
TFS1	10 (0–27)
TL2	9 (4–27)
TLS2	7 (2–16)
TL3	3 (0–11)
TM2	3 (0–8)

Note: Me, median.

In lung adenocarcinoma (LUAD), the number of THCs carrying markers of leukocytes (TL2 and TL3 subpopulations) and macrophages (TM2), as well as THCs with stem and leukocyte features (TLS2, TLS3) were higher than in lung squamous cell carcinoma (LUSC; $p < 0.05$, Table 5). In addition, THCs carrying leukocyte (TL2, TL3) and macrophage markers (TM2) and THCs with stem and leukocyte features (TLS2) were higher in NSCLC patients without neoadjuvant chemotherapy (NACT) than in therapy-naïve cases ($p < 0.05$, Table 5). Interestingly, THCs with leukocyte (TL2) and macrophage markers (TM2, TM1) and THCs carrying stem and leukocyte markers (TLS2) were lower in smokers than in non-smoking patients ($p < 0.05$, Table 5).

Table 5

Tumor hybrid cells number in NSCLC depending on clinicopathologic parameters, neoadjuvant chemotherapy, distant metastasis, and recurrence

	TL2	TL3	TM2	TM1	TLS2	TLS3	TFS1
LUAD	16 (8–42)	5 (2–18)	5 (1–20)	27 (1–112)	12 (4–35)	5 (0–15)	0 (0–4)
LUSC	6 (2–11)	1 (0–4)	1 (0–3)	61 (6–160)	5 (2–10)	1 (0–3)	1 (0–4)
p	0.00315	0.0135	0.0018	0.592	0.0100	0.0349	0.464
NACT+	7 (2–10)	0 (0–2)	1 (0–3)	56 (6–133)	3 (1–9)	0 (0–5)	2 (0–9)
NACT–	14 (6–38)	5 (2–17)	5 (1–11)	28 (1–119)	10 (4–32)	4 (0–16)	0 (0–2)
p	0.0111	0.0006	0.0080	0.713	0.01142	0.0025	0.055
Smoking +	8 (4–13)	3 (0–9)	1 (0–5)	0 (0–6)	6 (0–35)	3 (0–9)	1 (0–32)
Smoking–	20 (14–58)	4 (0–9)	9 (3–30)	1 (1–4)	13 (2–94)	1 (0–5)	0 (0–20)
p	0.008	0.91	0.0112	0.0167	0.0087	0.43	0.051
MTS+	8 (5–21)	5 (1–17)	0 (0–0)	22 (6–114)	6 (3–18)	3 (0–92)	2 (0–31)
MTS–	9 (4–27)	2 (0–6)	0 (0–1)	51 (2–179)	8 (2–15)	1 (0–50)	15 (0–236)
p	0.667	0.065	0.285	0.703	0.681	0.050	0.0217
Rec+	11 (8–27)	0 (0–8)	0 (0–0)	2 (0–60)	9 (5–11)	0 (0–7)	0 (0–4)
Rec–	8 (4–25)	3 (0–11)	0 (0–1)	0 (0–9)	6 (2–19)	3 (0–10)	1 (0–4)
p	0.433	0.160	0.380	0.017	0.785	0.20	0.490

Note: LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; NACT, neoadjuvant chemotherapy; MTS, distant metastasis; Rec, locoregional recurrence; +/–, yes/no; p, significance level.

THC with leukocyte (TL1) and macrophage features (TM1) and THCs carrying leukocyte and macrophage and stem cell markers (TLS1 and TMS1) were frequently detected in NSCLC patients.

THC with fibroblast features (TF1) were often observed in smokers compared to non-smoking patients (84.6 % (22) versus 50.0 % (6); p = 0.024).

In NACT-treated patients, the frequency of THCs with leukocyte (TL3) and macrophage (TM3) features and THCs with stem, leukocyte and macrophage markers (TLS3, TLS4, TLS5, TMS2, TMS3) was lower than in patients without NACT (p < 0.05, Table 6). In contrast, THCs carrying markers of fibroblasts (TF3) and THCs with stem and fibroblast (TFS2) features was frequently detected in patients with NACT than in therapy-naïve cases (p < 0.05, Table 6).

Table 6

Tumor hybrid cells frequency in NSCLC depending on neoadjuvant chemotherapy

THC subpopulations	NACT+	NACT–	p
TL3	28.6 (10)	66.7 (10)	0.012
TM3	21.1 (4)	51.6 (16)	0.032
TLS3	29.4 (10)	62.5 (10)	0.026
TLS4	17.6 (3)	51.5 (17)	0.021
TLS5	8.3 (1)	50.0 (19)	0.01
TMS2	21.1 (4)	51.6 (16)	0.032
TMS3	10.0 (1)	47.5 (19)	0.03
TF3	65.2 (15)	18.5 (5)	0.01
TFS2	65.0 (13)	23.3 (7)	0.003

Note: NACT, neoadjuvant chemotherapy; p, significance level.

Tumor hybrid cells associated with NSCLC distant metastasis and recurrence

Patients with distant metastases demonstrated an increase in the number of THCs with stem and leukocyte features (TLS3) (p < 0.05, Table 5). In addition, TLS5, TMS3 and TMS4 subpopulations were most frequent in metastatic patients than in patients without metastases (p < 0.05, Fig. 2 A).

In contrast, patients without distant metastases demonstrated an increase in the number of THCs carrying stem cell and fibroblast markers (TFS1) (p < 0.05, Table 5). Non-metastatic patients also had a high frequency of TFS3 and TF4 THCs than cases with metastases (p < 0.05, Fig. 2 A).

Patients with locoregional recurrence demonstrated an increase in the number of THCs with macrophage markers (TM1; p < 0.05, Table 5). TM4, TLS4, and TMS6 subpopulations were also most frequent in recurrent patients than in cases without recurrence (p < 0.05, Fig. 2 B).

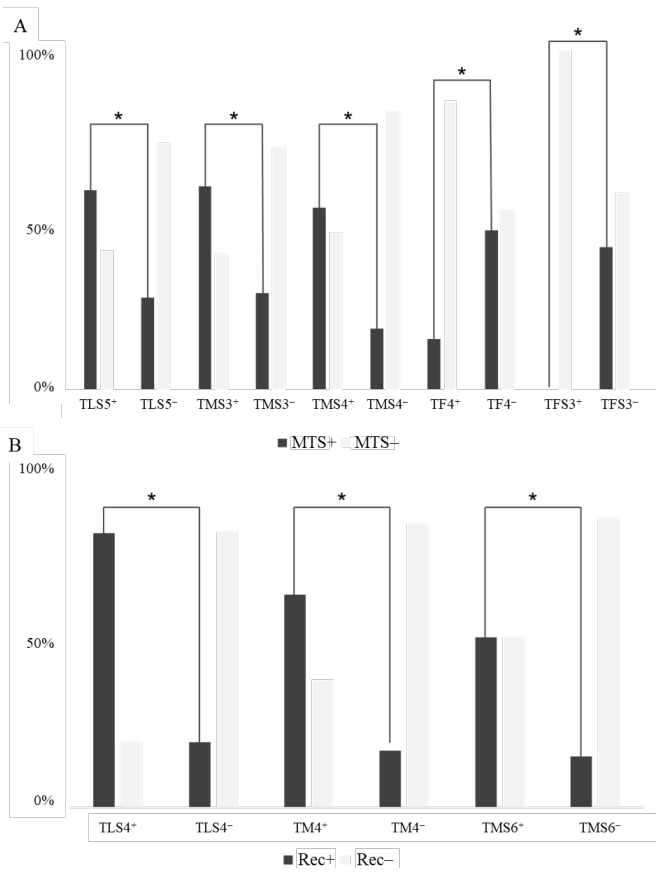

Figure 2. Frequency of distant metastasis (A) and recurrence (B) in NSCLC patients with (+) and without (–) different THC populations

Note: *, p < 0.05.

It is important to note that the TMS3 subpopulation was an independent predictor of distant metastases, whereas TLS4 — locoregional recurrence when taking into account clinicopathological parameters. At the same time, the prognostic significance of the TMS4 and TLS5 subpopulations remained only for male patients

(TMS4, p = 0.041) and highly graded tumors (TLS5, p = 0.033) (data not shown).

Tumor hybrid cells associated with survival of NSCLC patients

Poor overall survival (OS) rates were associated with THCs carrying stem and macrophage markers (TMS3 and TMS5) and THCs with stem and leukocyte features (TLS6) ($p < 0.05$, Fig. 3).

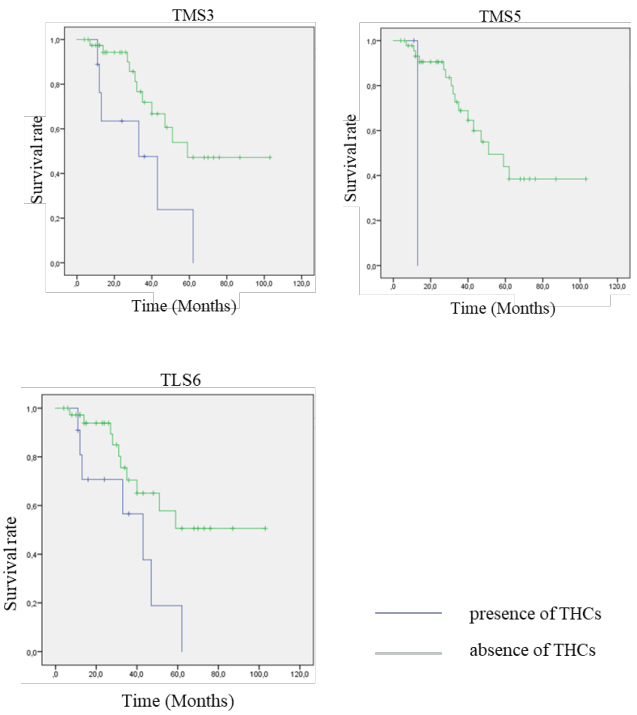

Figure 3. Overall survival of NSCLC patients depending on the presence of different tumor hybrid cells populations

Note: TMS3, $EpCAM^+CD45^+CD44^+CD73^+CD163^+$; TMS5, $pan\text{-}CK^+EpCAM^+CD45^+CD44^+CD73^+CD68^+CD163^+$; TLS6, $EpCAM^+CD45^+CD44^+CD73^+$.

Considering clinicopathological characteristics, the prognostic significance of TMS3 was valid only for grade 2 (p = 0.021), TMS5 — smokers (p = 0.011), TLS6 — LUAD (p = 0.032) and TMS4 — male smokers (p = 0.042).

Despite significant advances in NSCLC diagnosis of and therapy, the prevention of metastasis and recurrence still remains an unresolved problem. Even with timely diagnosis, most patients demonstrate disease progression in the postoperative period [28–30]. In this regard, the identification of metastasis- and recurrence-initiating cells and the development of approaches for their elimination is a priority task of modern oncology. A number of studies have shown that THCs may be one of the potential players in metastasis and recurrence [17, 18, 31]. Here, we first describe population composition of THCs in NSCLC and analyze their association with clinicopathological characteristics, metastasis and recurrence.

Regardless of the histological type, the majority of THCs harbor leukocyte and macrophage features. However, the number of THCs with markers of leukocytes and macrophages and THCs with stem features is significantly higher in LUAD than in LUSC. The number and frequency of THCs depend on NACT. Hybrid cells with leukocyte and macrophage characteristics and THCs with stem, leukocyte and macrophage markers are prevalent in patients without NACT. Distant metastases and recurrence are associated with THCs. Hybrid cells carrying stem and macrophage markers ($EpCAM^+CD45^+CD44^+CD73^+CD163^+$) are associated with distant metastases regardless of clinicopathological characteristics. Locoregional recurrence is associated with THCs with stem and leukocyte features ($pan\text{-}CK^+CD45^+CD44^+CD73^+$).

Previous study showed that NSCLC patients have giant $KRT8/18/19^+$ or $EpCAM^+$ and $CD14^+CD45^+$ hybrid cells of macrophage origin in the bloodstream, which are associated with shorter overall and disease-free survival [32]. Another study demonstrated that merging of mesenchymal stem cells and lung cancer cells leads to the formation of THCs with stem and epithelial-mesenchymal transition features and increased motility [33]. It has been also shown that cancer cells fuse with M2 macrophages, and such cells demonstrate stem-like characteristics ($CD44^+CD24^-$) and contribute to breast cancer metastasis [34].

However, the main limitation of this study is the use of a limited set of markers, which does not allow the phenotypic diversity of THCs to be fully characterized. Accordingly, future research should be based on the use of high-throughput methods, for example, single-cell sequencing, as shown previously in other cancers [35, 36], which will allow not only to assess the diversity of THCs in detail, but also to

understand the mechanisms of their formation and interaction with other tumor, immune and stromal cells.

Conclusion

Taken together, this study provides a detailed characterization of THC population composition in NSCLC that depends on neoadjuvant chemotherapy and is associated with locoregional recurrence and distant metastases.

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Опухолевые гибридные клетки при немелкоклеточном раке лёгкого: популяционный состав и клиническая значимость

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Аннотация. *Актуальность*. Немелкоклеточный рак легкого (НМРЛ) является одним из наиболее распространенных злокачественных новообразований. Основными причинами смертности от НМРЛ являются рецидивы и отдаленные

метастазы. Принято считать, что метастазы и рецидивы формируются опухолевыми клетками, обладающими высоким инвазивным и химиорезистентным фенотипом. По последним данным, такими клетками могут быть опухолевые гибридные клетки, формирующиеся в результате слияния опухолевых клеток с широким спектром нормальных клеток: макрофагами, фибробластами, мезенхимальными, стволовыми клетками и т.д. Однако состав, фенотипическое разнообразие ОГК и их связь с клинико-патологическими параметрами и прогрессированием НМРЛ остаются плохо изученными. Цель настоящего исследования — охарактеризовать популяционный состав опухолевых гибридных клеток при НМРЛ и его связь с клинико-патологическими параметрами, метастазированием и рецидивированием. Материалы и методы. В исследование было включено 50 пациентов с НМРЛ. Использовались морфологически верифицированные свежезамороженные образцы опухолевой ткани, полученные при резекции легкого по поводу НМРЛ. Опухолевые гибридные клетки анализировали методом проточной цитофлуориметрии с использованием маркеров опухолевых клеток, опухолевых стволовых клеток, лейкоцитов, макрофагов и фибробластов. Результаты и обсуждение. Опухолевые гибридные клетки были обнаружены у всех пациентов НМРЛ. Большинство опухолевых гибридных клеток были с лейкоцитарными, макрофагальными и стволовыми признаками. Количество и частота опухолевых гибридных клеток зависели от неоадъювантной химиотерапии. Опухолевые гибридные клетки с маркерами стволовости и лейкоцитов (pan-CK$^+$CD45$^+$CD44$^+$CD73$^+$) были связаны с локорегионарными рецидивами, тогда как ОГК с маркерами стволовости и макрофагов (EpCAM$^+$CD45$^+$CD44$^+$CD73$^+$CD163$^+$) — с гематогенными метастазами. Выводы. Впервые комплексно описан популяционный состав опухолевых гибридных клеток при НМРЛ и его ассоциация с клинико-патологическими характеристиками, неоадъювантной химиотерапией и прогнозом. Выявление прогностически значимых опухолевых гибридных клеток может быть потенциальным подходом для предсказания риска метастазирования и рецидивирования НМРЛ и основанием для подбора терапии, направленной на снижение вероятности прогрессирования данного заболевания.

Ключевые слова: опухолевые гибридные клетки, немелкоклеточный рак легкого, проточная цитофлуориметрия, метастазирование, рецидивирование

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