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Expression of chemerin and B7 family proteins in lung adenocarcinoma — pilot study

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ABSTRACT

Introduction: Lung cancer is the most commonly diagnosed cancer with 2.2 million cases in 2020 and causes 1.8 million deaths. Early lung cancer often has no symptoms and can only be detected by medical imaging. When symptoms do appear, they are often respiratory problems —coughing, breathlessness or chest pain — and systemic problems — loss of appetite, weight loss, general weakness, fever and night sweats. There are two main types of lung cancer: small cell lung cancer (SCLC; 15% of cases) and non-small cell lung cancer (NSCLC; 85% of cases).

Material and methods: This study included evaluation of CMKLR1, PD-1, B7H3, B7H4 and HHLA2 expression, along with CD8 + T-cell population, TILs and budding in H + E stained slides using IHC. Although there was no clear association between the analysed expressions and the T parameter, this study, which included 22 archived lung adenocarcinoma cases from patients undergoing radical lobectomy, revealed significant negative correlations between HHLA2 expression and tumour grade, as well as between CMKLR1 expression and tumour grade.

Results: Furthermore, CMKLR1 expression among lymphocytes showed a positive correlation with TILs.

Keywords: NSCLC, immunohistochemical expression, B7H3, TILs, CMKLR1

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Introduction

Lung carcinoma is one of the malignancies with the highest incidence rate, furthermore, it is the leading cause of cancer mortality worldwide for both sexes [1]. Continuous exposure to tobacco smoke is mostly blamed for lung cancer. However, the proportion of smoking patients to non-smokers is changing. Lung adenocarcinoma has a strong association with previous smoking, but the group of patients who were not exposed to tobacco smoke is increasing [2]. Studies report genetic factors as the main risk factor for non-smokers with a family history of cancer. Other risk factors mentioned in the studies are environmental air pollution, passive smoking and asthma, pneumonia, chronic bronchitis, and viral infection as a group of respiratory diseases.

The immune system plays a crucial role in cancer pathogenesis and treatment. Lung carcinoma development is related to chronic inflammation. Immune cells can produce several cytokines that may contribute to both tumour development and suppression. Other important factors in tumour pathogenesis are adipokines, initially described to be expressed in adipose tissue. Further research showed that adipokines may be produced in many other tissues as well as in malignant tumour cells [3, 4].

The topic of this study is the expression and distribution of chemerin and its receptor, CMKLR1. Both are involved in the pathogenesis of many malignancies, especially known for their role as pro-angiogenic factors. Additionally, CMKLR-chemerin signalization may contribute to the epithelial-mesenchymal transition of tumour cells that may contribute to tumour spread [5–7].

Homeostasis of the immune system depends on a balance between excitatory and inhibitory signals. This study focused on selected proteins that appear to influence the course of disease in diagnosed patients. Programmed death ligand (PD-L1) is a molecule responsible for the impaired immune system targeting programmed death receptor 1 (PD-1) [8].

Also included in the study is the B7 family of proteins (B7H3, B7H4 and HHLA2) which are essential for regulating T lymphocyte responses. Members of the B7 family function as immune regulatory ligands that interact with the CD28 family of receptors. B7H3 (CD276) is a newly discovered type 1 trans-membrane glycoprotein used in cancer immunotherapy as an immune checkpoint.

The B7 homolog 4 (B7-H4) encoded by the VTCN1 gene is the seventh member of the B7 family of cell signalling ligands and is highly expressed in tumours [9]. Additionally, B7H4 was found to regulate the innate and adaptive immune system, suggesting its immunosuppressive capacity.

Human endogenous retrovirus-H long terminal repeat-associating protein 2 (HHLA2) is another member of the B7 family, whose expression is upregulated in multiple tumours. However, its role in NSCLC has not been fully understood.

For leukocyte populations expressing the G protein-coupled receptor CMKLR1 (ChemR23), chemerin appears to act as a chemotactic factor [6]. The orphan GPCR receptor ChemR23 shares homology with neuropeptide and chemoattractant receptors and is expressed in monocyte-derived dendritic cells, macrophages, antigen-presenting cells and, at a lower level of expression, $CD4^+$ T lymphocytes [10]. As a result, orphan G protein-coupled receptors (GPCRs) have been successfully used in reverse pharmacology screening to identify potential new drug targets.

Hence, this study aimed to assess the immunohistochemical expression of CMKLR1, chemerin, HHLA2 B7H3 and B7H4 in association with the adenocarcinoma subtype and other factors including, tumour-infiltrating lymphocytes (TILs) and clinicopathological parameters such as tumour stage based on World Health Organization (WHO) classification, tumour grading and histological pattern analysis.

Material and methods

The study involved 22 archival cases of lung adenocarcinoma from patients who underwent radical lobectomy between May 2018 and December 2021.

Table 1. General characteristics of study group

Sociodemographic data	
Age [mean (SD)]	**63.68 (6.64)**
Males (%)	14 (63.6)
Females (%)	8 (36.4)
Tumour characteristics	
T parameter	
1a	1 (4.5)
1b	4 (18.2)
1c	4 (18.2)
2a	6 (27.3)
2b	1 (4.5)
3	4 (18.2)
4	2 (9.1)
N parameter	
0	19 (86.4)
1	2 (9.1)
2	1 (4.5)
Lymphatic vessel invasion (%)	6 (27.3)
Vascular invasion (%)	5 (22.7)
Perineural invasion (%)	2 (9.1)
Pleural invasion	
Pl0	17 (77.3)
Pl1	4 (18.2)
Pl2	1 (4.5)
Spread through air spaces (STAS)	10 (45.5)
Grade	
G1	0 (0)
G2	12 (54.5)
G3	10 (45.5)
Max tumour size (mean (SD))	37.95 (21.19)

The inclusion criteria were: histopathologically confirmed primary lung adenocarcinoma.

The exclusion criteria were: histopathologically confirmed adenosquamous carcinoma, secondary lung neoplasm, a specific type of adenocarcinoma (colloid/ foetal/ enteric-type), occurrence of more than one histologically different tumour in post-operative material and occurrence of distant metastases.

Clinico-pathological characteristics of the study group are presented in Table 1.

Table 2 presents the percentage distribution of tissue within the tumour.

Table 2. Percentage distribution of tissue within the tumour

Histologic pattern	Mean (SD)
Lepidic	25 (37.5)
Acinar	60 (46.2)
Papillary	7.5 (2.5)
Solid	75 (32.5)

Immunostaining

Histopathologic examination

For PD-L1, CMKLR1, chemerin, HHLA2 B7H3 and B7H4 staining specimens were assessed by two independent pathologists. In each case, the percentage of stained cells was assessed as well as staining intensity, defined as low (+), medium (++) and high (+++). In the next step, the Expression level was calculated as the percentage of stained cells multiplicated by staining intensity and divided by 300 to obtain results in the 0–1 Range, where 0 means no expression, while 1 refers to strong expression in every cell. These scores were calculated independently for tumour cells, tumour infiltrating lymphocytes (TILs) and lung tissue.

The percentage of tumour-associated lymphatic infiltration was estimated semi-quantitatively on a five-tier scale on the same H&E-stained slides by the two pathologists, according to the criteria defined by Salgado et al. in breast cancer. These include intratumoral lymphocytes with cell-to-cell contact between lymphocyte and tumour cells and stromal TILs in tumour tissue located dispersed in the stroma within the tumour cells without direct contact, including TILs at the invasive margin. According to the recommendations, stromal TILs were scored as a percentage of the stromal area alone, excluding areas occupied by carcinoma cells. Lymphocytic infiltrates outside the tumour borders were not included in the evaluation. Lymphocyte infiltration area lower than 5% was considered TILs 1, whereas 5–25%, 25–50%, and 50–75% of lymphocytes in the stroma were defined as TILs 2, TILs 3 and TILs 4, respectively. More than 75% was defined as TILs 5.

CD3 and CD20 assessment

CD3 and CD20 were assessed as a percentage of total tumour lymphocytic infiltration.

Results

Expression of examined molecules among specimens is presented in Table 3. Table 4 presents the percentage of positively stained specimens in the study group. All examined tumours were chemerin-positive. Chemerin receptor (CMKLR1) was positively expressed among 77.3% of tumour specimens while lung tissue expression occurred only in 54.5% of patients.

Association between molecule expression and clinicopathological parameters.

No significant correlation between examined expressions and the T parameter was found. However, a significant negative correlation was found between HHLA2 expression and tumour grade (Tau = –0.47, $p = 0.012$) as well as between CMKLR1 expression and tumour grade (Tau = –0.42, $p = 0.02$). CMKLR1 expression among lymphocytes was positively correlated with TILs (Tau = 0.34, $p = 0.04$).

HHLA 2 expression was additionally positively correlated with the per cent of the lepidic pattern (Tau = 0.51, $p < 0.01$) and negatively correlated with solid pattern (Tau = –0.49, $p < 0.01$) per cent while chemerin expression was positively correlated with the per cent of the lepidic pattern (Tau = 0.35, $p = 0.049$) and negatively with per cent of solid pattern (Tau = –0.37, $p = 0.036$).

No significant association between molecule expression and lymph node involvement, nor vascular invasion were found.

Discussion

CMKLR1 receptor was positively expressed among 77.3% of examined tumours (95% CI: 59.76–81.14%). To the best of the authors' knowledge, the presented article is the first one which assesses CMKLR expression in lung adenocarcinoma using immunohistochemical staining. Chemerin/CMKLR1 is known to play an important role in the angiogenesis process as well as in promoting the invasion of gastric cancer cells in vitro [6, 7, 11]. This study found a significant negative correlation between CMKLR1 expression and tumour grade.

Additionally, a positive correlation was found between CMKLR expression among lymphocytes and tumour lymphocytic infiltration intensity. The Chemerin/CMKLR1 axis is known to play a role in the suppression of tumour growth via the recruitment of immune system cells into the tumour microenvironment. This phenomenon was observed in melanoma in vivo experiments on animals specifically mediated by NK cells [12].

Table 3. Expression of examined molecules among specimens

Variable	N (%)	Lower CI	Upper CI
Tumour			
PDL1	19 (86.4%)	72.02%	89.53%
CMKLR1	17 (77.3%)	59.76%	81.14%
Chemerin	22 (100.0%)	100.00%	100.00%
B7H4	10 (45.5%)	24.65%	50.06%
B7H3	17 (77.3%)	59.76%	81.14%
HHLA2	20 (90.9%)	78.90%	93.57%
Lymphocytes			
PDL1	21 (95.5%)	86.75%	97.38%
CMKLR1	20 (90.9%)	78.90%	93.57%
Chemerin	16 (72.7%)	54.12%	76.84%
HHLA2	21 (95.5%)	86.75%	97.38%
Lung tissue			
Chemerin	22 (100.0%)	100.00%	100.00%
CMKLR1	12 (54.5%)	33.74%	59.15%

Table 4. Quantitative expression of examined molecules

Tumour	Median [IQR]
Index PDL1 tumour (median [IQR])	0.05 [0.03, 0.50]
Index CMKLR1 tumour (median [IQR])	0,63 [0.06, 0.77]
Index CHM tumour (median [IQR])	0.53 [0.33, 0.67]
Index HHLA2 tumour (median [IQR])	0.47 [0.28, 0.67]
Index B7H4 tumour (median [IQR])	0.00 [0.00, 0.09]
Index B7H3 tumour (median [IQR])	0.10 [0.02, 0.27]
Lymphocytes	
Index CMKLR1 lymph (median [IQR])	0.35 [0.11, 0.67]
Index PDL1 lymph (median [IQR])	0.06 [0.03, 0.10]
Index CHM lymph (median [IQR])	0.04 [0.00, 0.18]
Index HHLA2 lymph (median [IQR])	0.17 [0.07, 0.40]
Lung tissue	
Index CMKLR1 lung (median [IQR])	0.10 [0.00, 0.28]
Index CHM lung (median [IQR])	0.33 [0.31, 0.67]

Studies on adrenocortical cancer showed that chemerin plays a role in tumour suppression and its high serum concentration is associated with better overall survival. Authors propose that chemerin expression may undergo downregulation caused by tumours to escape immune defences, while host systems are trying to upregulate chemerin to stimulate tumour response. This study found no correlation between chemerin expression and tumour size or T parameter. As aforementioned, a significant negative correlation between tumour grade and CMKLR1 expression was found, thus it may be concluded that high-grade tumours may have a weaker response to chemerin stimulation via the CMKLR1 receptor. Despite the lack of CMKLR1 expression in some tumours, chemerin was found to be expressed in every case in both lung tissue and malignant cells. There is evidence for both, stimulating and suppressor roles of chemerin in cancer pathogenesis. Additionally, chemerin is also well known as a regulator of immune response by stimulating macrophages and dendritic cells as well as by influencing lymphocyte migration [13].

Another important factor in the tumour-associated immune response is members of the B7 family that are involved in the regulation of T-cell-associated cytotoxicity, which is crucial in anti-tumoral response [14]. The B7 family contains such molecules as PD-L1 whose expression is already commonly used in histopathological assessment of lung carcinoma but also such molecules as B7-H3(CD276), B7-H4(vtcn1) and B7-H7(HHLA2). These molecules are responsible for T-supporting T-cell activity as well as its downregulation. B7H3 is found to

be expressed in such malignancies as breast cancer, colorectal cancer and lung cancer. B7H3 expression was found in 64.9% of cases by Hausdorf et al. using the immunofluorescence method [15]. These findings are close to the present results (77%, 95% CI: 59.76–81.14%), however, the same authors found B7-H4 expression in 2.6% of cases, while in the present study B7H4 positive tumoral expression was found in 45.5% of examined specimens, however, it was low in most cases (h-score median 0.00; IQR 0.00:0.09) Altan et al. [15] reported positive expression of B7H4 in immunohistochemical staining among 50.5% of EGFR mutated lung carcinomas and 39.2% of wildtype lung adenocarcinoma. These results are similar to the present findings. Inamura et al. reported that B7-H3 expression is associated with tumour stage and grading [16]. In the following study, no correlation was found between B7-H3 expression and grade nor tumour stage in the case of B7-H3 and B7-H4 respectively. No associations between B7H4 nor B7H3 and clinicopathological parameters as well as with lymphatic infiltration were found. HHLA2 expression was found among 90.9% of specimens. Chen et al. reported positive HHLA2 expression in 100% of both, EGFR-mutated and wild-type lung carcinomas [17]. Upregulation of HHLA2 in lung carcinoma was also reported by Sun et al. [18]. Authors suggested that knockout of HHLA2 may inhibit lung carcinoma cell migration and invasion as well as suppress tumorigenesis in vivo. This study found a negative correlation between HHLA2 expression and tumour grade.

Article information

Data availability statement: *The data presented in this study are available in this article.*
Ethics statement: *The study was approved by the Bioethics Committee of the Medical University of Silesia in Katowice.*
Author contributions: *Conceived and designed the analysis: Paweł Kiczmer, Miriam Terenowicz; Collected the data: Miriam Terenowicz, Małgorzata Katra; Contributed the data: Sylwia Mielcarska, Mateusz Rydel, Damian Czyżewski; Performed the analysis: Paweł Kiczmer, Bogna Drozdowska; Wrote the paper: Paweł Ziora, Miriam Terenowicz, Paweł Kiczmer, Małgorzata Katra; Other contribution: Paweł Kiczmer, Miriam Terenowicz, Małgorzata Katra, Sylwia Mielcarska, Paweł Ziora, Mateusz Rydel, Damian Czyżewski, Bogna Drozdzowska.*
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