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Clinical significance of long non-coding RNA expression in esophageal cancer depending on the phenotype of the tumor stroma

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Introduction. Esophageal squamous cell carcinoma is a dangerous oncological disease for which there are no relevant molecular-biological and biochemical markers for diagnosis, monitoring, and prognosis. Non-coding RNAs, whose aberrant expression is characteristic of many neoplasms may be promising candidate markers.

Aim. To investigate the clinical significance of the expression of long non-coding RNAs (lncRNAs) SNGH18, LCAL1, IGFL2-AS1, LINC02301 and LINC01508 in esophageal squamous cell carcinoma depending on the phenotype of the tumor stroma.

Materials and methods. The study included 17 patients with esophageal squamous cell carcinoma, who were examined and treated at the N.N. Blokhin National Medical Research Center of Oncology. Gene expression levels were assessed using real-time polymerase chain reaction. Immunohistochemical analysis was conducted to evaluate the expression of CD68, CD163 and inducible nitric oxide synthase. Statistical analysis of the obtained results was performed using GraphPad Prizm v. 10. Differences in lncRNA expression between tumor samples and conditionally normal tissues were assessed using the Wilcoxon test for paired samples. Correlation analysis was carried out by calculating Spearman's correlation coefficient. Survival analysis was conducted using Kaplan–Meier survival curves. A *p*-value of less than 0.05 was considered statistically significant.

Results. Aberrant expression of the lncRNAs LCAL1, LINC01508 and LINC02301 was observed in tumor tissue compared to conditionally normal tissue. Specifically, the expression of LCAL1 and LINC01508 was increased in tumor tissue ($p = 0{,}001$ and $p = 0{,}007$), while the expression of lncRNA LINC02301 was decreased ($p = 0{,}004$). The expression of lncRNAs SNGH18 and IGFL2-AS1 showed no significant changes. ROC-analysis indicated that examining these lncRNA expressions is not suitable for diagnosing esophageal squamous cell carcinoma. Clinical significance analysis revealed no correlation between the studied lncRNA expressions and the clinicopathological characteristics of the disease correlation analysis of the lncRNAs SNGH18, LCAL1, IGFL2-AS1, LINC02301 and LINC01508 with the phenotype of tumor stroma macrophages demonstrated that LINC01508 was significantly and positively correlated with both the total number of macrophages ($r = 0.579$; $p = 0.017$) and the number of macrophages with cytotoxic and immunosuppressive phenotypes ($r = 0.567$; $p = 0.004$ and $r = 0.496$; $p = 0.045$, accordingly). In contrast, LCAL1 expression was inversely correlated with the number of cytotoxic macrophages ($r = -0.490$; $p = 0.037$). Prognostic analysis revealed that only lncRNA IGFL2-AS1 expression was associated with favorable prognosis in esophageal squamous cell carcinoma (hazard ratio 0.374; $p = 0.039$).

Conclusion. Long non-coding RNAs are important regulatory elements in both normal and tumor cells, offering certain advantages for the diagnosis of oncological diseases due to their high specificity and stability in both tissues and circulating body fluids. Growing evidence from scientific research highlights the potential clinical application of lncRNA expression analysis as markers for early diagnosis and as potential therapeutic targets. In this study, we conducted a retrospective investigation and determined the clinical significance of lncRNAs SNGH18, LCAL1, IGFL2-AS1, LINC02301, LINC01508 in esophageal squamous cell carcinoma, thereby expanding our understanding of the molecular changes observed in the development of this disease.

Keywords: esophageal squamous cell carcinoma, non-coding RNAs, lncRNA, microenvironment, macrophages

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Клиническая значимость экспрессии длинных некодирующих РНК при раке пищевода в зависимости от фенотипа опухолевой стромы

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Введение. Плоскоклеточный рак пищевода является опасным онкологическим заболеванием, для которого нет релевантных молекулярно-биологических и биохимических маркеров диагностики, мониторинга и прогноза. Одними из перспективных маркеров могут выступать некодирующие РНК, аберрантная экспрессия которых характерна для многих новообразований.

Цель исследования – изучение клинической значимости экспрессии длинных некодирующих РНК (днРНК) SNGH18, LCAL1, IGFL2-AS1, LINC02301 и LINC01508 при плоскоклеточном раке пищевода в зависимости от фенотипа опухолевой стромы.

Материалы и методы. В ретроспективное исследование включены 17 пациентов с плоскоклеточным раком пищевода, проходивших обследование и лечение в Национальном медицинском исследовательском центре онкологии им. Н.Н. Блохина. Уровень экспрессии генов исследуемых днРНК оценивали с помощью полимеразной цепной реакции в реальном времени. Иммуногистохимическим методом проведен анализ экспрессии CD68, CD163 и индуцибельной синтазы оксида азота. Статистический анализ полученных результатов выполняли с использованием программного решения GraphPad Prizm v. 10. Различия экспрессии днРНК между образцами опухолей и условно нормальных тканей оценивали с помощью критерия Уилкоксона для парных выборок. Корреляционный анализ проводили посредством определения коэффициента ранговой корреляции Спирмена. Выживаемость анализировали путем построения кривых дожития по методу Каплана–Майера. Различия считались статистически значимыми при p <0,05.

Результаты. Выявлено, что для днРНК LCAL1, LINC01508 и LINC02301 наблюдалась аберрантная экспрессия в опухолевой ткани по сравнению с условной нормой. Так, в опухолевой ткани экспрессия LCAL1 и LINC01508 оказалась повышенной (p = 0,001 и p = 0,007), в то время как для LINC02301 наблюдалось снижение экспрессии (p = 0,004). Экспрессия днРНК SNGH18 и IGFL2-AS1 значимо не изменялась. Результаты ROC-анализа продемонстрировали, что исследование экспрессии данных днРНК не подходит для диагностики плоскоклеточного рака пищевода. Анализ клинической значимости экспрессии изучаемых днРНК показал, что этот параметр не коррелирует с клинико-морфологическими характеристиками заболевания. Корреляционный анализ экспрессии днРНК SNGH18, LCAL1, IGFL2-AS1, LINC02301 и LINC01508 с фенотипом макрофагов стромы опухоли продемонстрировал, что днРНК LINC01508 значимо прямо коррелирует с содержанием как общего числа макрофагов (r = 0,579; p = 0,017), так и макрофагов цитотоксического и иммуносупрессорного фенотипов (r = 0,567; p = 0,004 и r = 0,496; p = 0,045 соответственно), а экспрессия LCAL1 обратно коррелирует с содержанием цитотоксических макрофагов (r = –0,490; p = 0,037). Анализ прогностической значимости показал, что только экспрессия днРНК IGFL2-AS1 является фактором благоприятного прогноза при плоскоклеточном раке пищевода (отношение рисков 0,374; p = 0,039).

Заключение. Длинные некодирующие РНК являются важными регуляторными элементами в нормальных и опухолевых клетках и обладают определенными преимуществами при диагностике онкологических заболеваний благодаря своей высокой специфичности и стабильности как в тканях, так и в циркулирующих жидкостях организма. Все больше данных, полученных в ходе научных исследований, показывают перспективы потенциального клинического применения анализа экспрессии днРНК в качестве маркеров ранней диагностики и потенциальных терапевтических мишеней. Мы провели ретроспективное исследование и определили клиническую значимость днРНК SNGH18, LCAL1, IGFL2-AS1, LINC02301, LINC01508 при плоскоклеточном раке пищевода, что расширяет наши представления о молекулярных изменениях, наблюдаемых при развитии данного заболевания.

Ключевые слова: плоскоклеточный рак пищевода, некодирующие РНК, длинные некодирующие РНК, микроокружение, макрофаг

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INTRODUCTION

Esophagus squamous cell carcinoma (ESCC) is one of the most common types of malignant tumors of the digestive tract, characterized by a low 5-year survival rate. In its early stages, the disease is often asymptomatic, resulting in late diagnosis and poor outcomes. Understanding the mechanisms of tumor development and progression in this condition contributes to the identification of new diagnostic markers and therapeutic targets. The literature describes numerous potential diagnostic and prognostic

markers for esophageal cancer, as well as their combinations for early diagnosis and disease monitoring [1–3]. However, the available data remain insufficient for successful clinical application.

It is well known that epigenetic factors, such as DNA methylation, histone modifications, chromatin remodeling, and regulatory non-coding RNAs (ncRNAs) play a significant role in tumor progression. The primary types of these ncRNAs are long non-coding RNAs (lncRNAs) and microRNAs. In recent years, ncRNAs have increasingly been recognized as potential diagnostic markers for various cancers, as their expression levels differ between normal and tumor tissues. Furthermore, they are regarded as markers for monitoring and predicting disease progression [4]. Another important factor in tumor pathogenesis is the tumor microenvironment, particularly tumor-associated macrophages, which represent the primary population of inflammatory infiltrating cells.

In esophageal cancer, as with certain other types of gastrointestinal tumors, a high presence of tumor-associated macrophages in the tumor stroma has been shown to have favorable prognostic significance, which sets this type of cancer apart from most other malignancies [5]. It is believed that tumor-associated macrophages, depending on their phenotype, can either promote tumor progression or inhibit its development [6]. However, this paradigm has recently shifted, with new evidence suggesting that all macrophages within the tumor, regardless of their phenotype, may contribute to its progression. Specifically, the cytotoxic activity of macrophages may lead to the selection of more malignant cell clones that are resistant to macrophage cytotoxicity, thereby promoting tumor spread. In previous studies on prostate and lung tumor models, we identified certain lncRNAs whose expression changes were observed during the process of cell resistance to cytotoxic activity [7].

Aim. To investigate the clinical significance of the expression of lncRNAs SNGH18, LCAL1, IGFL2-AS1, LINC02301, and LINC01508 in squamous cell carcinoma of the esophagus, depending on the phenotype of the tumor stroma.

MATERIALS AND METHODS

A retrospective study included 17 patients with ESCC at various stages of tumor progression, who underwent examination and treatment at the N.N. Blokhin National Medical Research Center of Oncology. All procedures performed in the study involving patients complied with the ethical standards of the organization's ethics committee and the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed voluntary consent was obtained from each participant included in the study. The clinical diagnosis of all patients was confirmed by morphological examination of the tumor in accordance with the International Histological Classification of Tumors of the Digestive System (World Health Organization, 2019). A description of the studied sample is provided in table 1.

Table 1. *Clinical and morphological characteristics of esophagus squamous cell carcinoma patients*

Parameter	Number of cases, abs. (%)
Age, years:	
≤60	8 (47)
>60	9 (53)
Gender:	
male	15 (88)
female	2 (12)
Stage:	
I–II	7 (41)
III–IV	10 (59)
Tumor size:	
T2–3	12 (71)
T4	5 (29)
Nodal status:	
N0	8 (47)
N+	9 (53)
Metastasis:	
M0	14 (82)
M+	3 (18)
Grade:	
G_{1-2}	11 (65)
G_3	6 (35)

The immunohistochemical study was conducted using standard techniques on sections of formalin fixed paraffin embedded tumor tissue. Antigen retrieval was performed using a Tris-EDTA buffer with a pH of 9.0. Primary antibodies to CD163 (clone 10D6; BIOCARE, 1:100), inducible nitric oxide synthase (iNOS) (SAB5500152; Sigma, 1:150), and CD68 (clone GR021, 61-0184 Genemed, 1:100) were applied to the sections and incubated for 60 minutes. The UltraVision Quanto Detection System HRP DAB (Thermo Scientific) universal two-component detection system was used, following the manufacturer's instructions. The prepared slides were evaluated under an OLYMPUS BX53 microscope at 200x magnification. The overall expression of CD163, iNOS, and CD68 in the tumor stroma was scored based on the percentage of antigen-positive cells: 0 – <1 %; 1 – <25 %; 2 – 25–50 %; 3 – >50 %. For further analysis, groups were classified into low-expression (0–1) and high-expression (2–3) categories.

Total RNA was isolated from the cells using the RNeasy Kit (Qiagen) in combination with the Trizol reagent following the standard protocol. Complementary DNA (cDNA) synthesis was performed through reverse transcription using the RevertAid RT Kit (Thermo Scientific). Gene expression levels were assessed by real-time polymerase chain reaction. Primer sequences are provided in table 2. Amplification was carried out on a CFX96 Touch amplifier (Bio-Rad) with the following thermal cycling program: 95 °C for 5 minutes, followed by 40 cycles of 95 °C

Table 2. *Primer sequences*

Gene	Forward primer	Reverse primer	t, °C
SNGH18	5' CCTAATGCTAAACATTGGTACA 3'	5' GCAACACAGCATCACCTGTAC 3'	60
LCAL1	5' TCCAGCTACCTGCCACTTGC 3'	5' TGCCAACTGCTTGTTCACCTG 3'	60
IGFL2-AS1	5'AGCCTATTTCCAGACAACT 3'	5'AGAATCAACGACCTCTACAT 3'	53
LINC01508	5' GTATGGGGTGTCTAATCAGGG 3'	5' GACTGTGGCTTTGCTAATGG 3'	57
LINC02301	5' TCTGGGCAGAAGGATCTACG 3'	5'AGCATCTAGGCCATGTGACC 3'	60
GAPDH	5' TCGGAGTCAACGGATTTGGT 3'	5' TCCCGTTCTCAGCCTTGACG 3'	60

for 10 seconds, 60 °C for 30 seconds, and 72 °C for 30 seconds. All reactions were conducted in two independent biological replicates. Data analysis was performed using the Bio-Rad CFX Manager software. The housekeeping gene GAPDH was used as the reference gene. The relative gene expression levels were calculated using the fold change (FC) method with the formulas:

$$\Delta Ct = Ct\ (\text{gene of interest}) - Ct\ (GAPDH),$$
$$\Delta\Delta Ct = \Delta Ct\ (\text{sample}) - \Delta Ct\ (\text{control}),$$
$$FC = 2^{-(\Delta\Delta Ct)}.$$

Statistical analysis of the obtained results was performed using GraphPad Prism v. 10. Differences in lncRNA expression between tumor samples and conditionally normal tissues were assessed using the Wilcoxon test for paired samples. The diagnostic method's informativeness, including its sensitivity and specificity, was analyzed by constructing ROC curves and calculating the area under the curve (AUC). Correlation analysis was conducted using Spearman's correlation coefficient. Survival analysis was performed by constructing Kaplan–Meier survival curves. The follow-up period was the time from surgery to the patient's death or their last medical visit. For the analysis of long-term treatment outcomes, patients were divided into two comparison groups based on the relative level of lncRNA expression, either above or below the median. The significance of differences was compared using the log-rank test. To assess the potential impact of various risk factors on survival, multivariate analysis was additionally performed using Cox's proportional hazards model. Differences were considered statistically significant at $p < 0.05$.

RESULTS

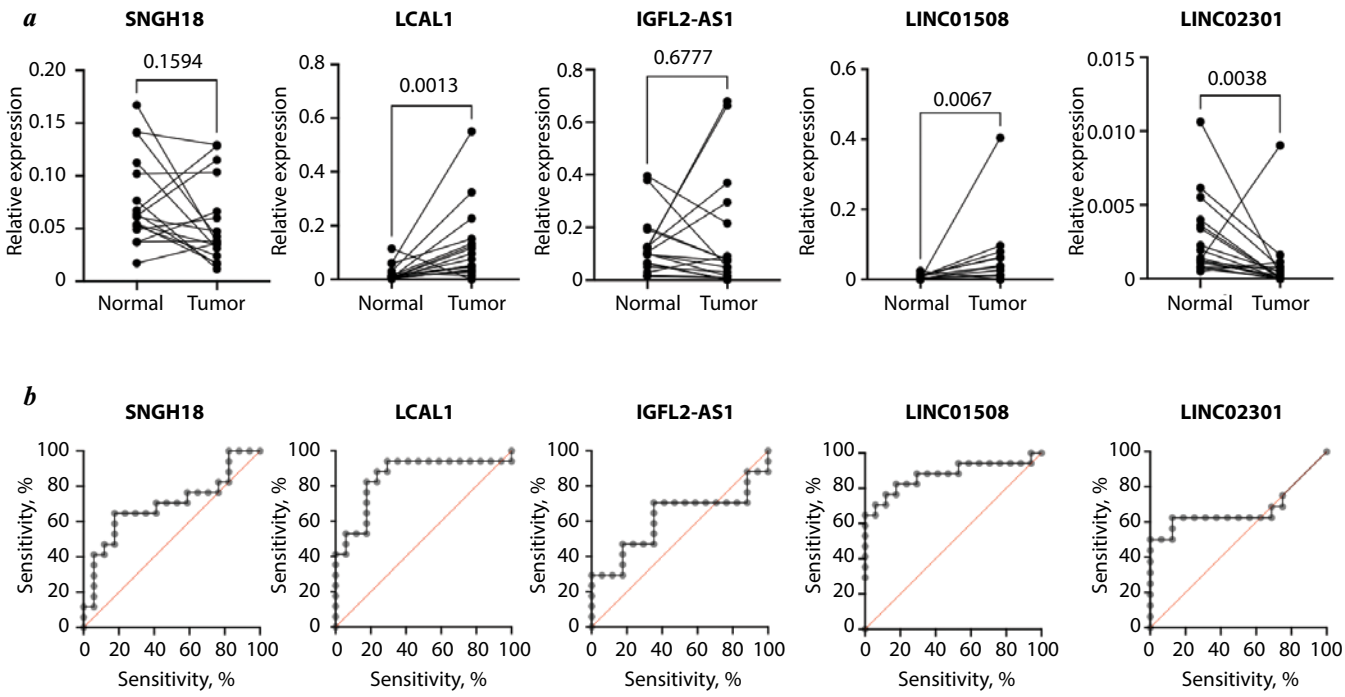
In the first stage of the study, the expression levels of the investigated lncRNAs were evaluated in both tumor tissue and conditionally normal esophageal tissue. Subsequently, the diagnostic method's informativeness was analyzed by assessing its sensitivity and specificity through the construction of ROC curves and the calculation of the AUC. The results are presented in fig. 1.

The analysis revealed that the investigated lncRNAs exhibited aberrant expression in esophageal tumors compared to normal tissue, with the changes being bidirectional. Specifically, LCAL1 and LINC01508 lncRNAs demonstrated significantly higher expression in tumor tissue, while LINC02301 showed a decrease in expression. The expression levels of SNGH18 and IGFL2-AS1 lncRNAs did not change significantly (fig. 1, *a*). ROC analysis indicated a trend, suggesting the potential diagnostic value of these lncRNAs, but highlighting the need for further analysis on a larger patient sample to confirm these findings (fig. 1, *b*).

Next, a correlation analysis was conducted to examine the relationship between the expression of the studied lncRNAs and the clinicopathological characteristics of the disease. The results of this analysis are presented in table 3.

As shown in the data presented in table 3, the expression levels of the lncRNAs SNGH18, LCAL1, IGFL2-AS1, LINC02301, and LINC01508 did not demonstrate any significant correlation with the clinicopathological characteristics of the disease.

In the next phase of the study, we assessed the prognostic significance of the investigated lncRNAs. The results of this analysis are shown in fig. 2.



*Statistically significant.

Fig. 1. *Expression analysis of SNGH18, LCAL1, IGFL2-AS1, LINC01508, LINC02301 long non-coding RNA in ESCC: a – comparative analysis of SNGH18, LCAL1, IGFL2-AS1, LINC01508, LINC02301 long non-coding RNA expression in conditionally normal and tumor tissue of esophagus. Analysis was performed using the Wilcoxon test. The graphs show the p-value; b – ROC analysis for SNGH18, LCAL1, IGFL2-AS1, LINC01508, LINC02301 in esophagus squamous cell carcinoma patients – SNGH18, area under the ROC curve (AUC) is 0.696 (p = 0.052), LCAL1, AUC is 0.851 (0.0005*), IGFL2-AS1, AUC is 0.613 (p = 0.263), LINC01508AUC is 0.676 (p = 0.089), LINC02301, AUC is 0.875 (p = 0.0002*)*

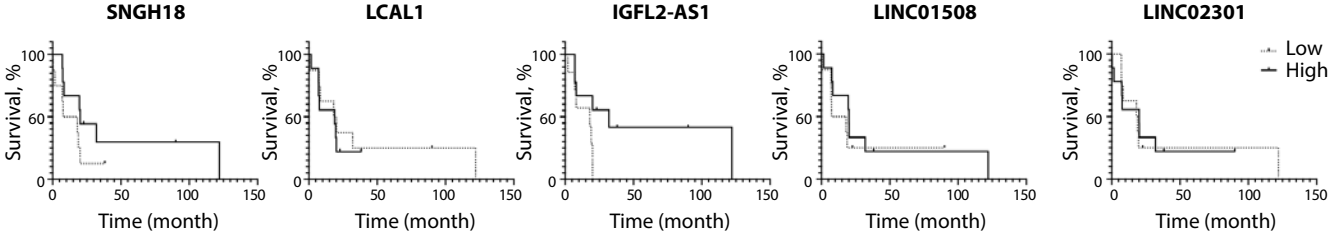


Fig. 2. *Analysis of overall survival depending on the expression of SNGH18, LCAL1, IGFL2-AS1, LINC01508, LINC02301 in esophagus squamous cell carcinoma tumor tissue*

The results of the study indicated that, with the exception of IGFL2-AS1, the expression of the investigated lncRNAs is not a prognostically significant factor in esophageal squamous cell carcinoma (ESCC). High expression of IGFL2-AS1 was found to be a reliable indicator of favorable prognosis. Specifically, patients with low IGFL2-AS1 expression had a median survival of 18 months, while those with high expression had a median survival of 32 months.

Subsequently, we conducted both univariate and multivariate analyses regarding patient survival based on lncRNA expression levels. The results are presented in table 4.

Given that the clinical significance of tumor-associated macrophages in esophageal tumors has not been definitively established, and the investigated lncRNAs are associated with tumor cells acquiring resistance to the cytotoxic activity of macrophages, the next phase of our work involved an immunohistochemical assessment of the expression of macrophage markers CD68, CD163, and iNOS in tumor samples.

We analyzed the clinical and prognostic significance of tumor infiltration by macrophages of various phenotypes. The presence of CD163+ macrophages was found to significantly inversely correlate with disease stage ($p = 0.05$). Additionally, only CD163+ macrophages showed a trend

Table 3. *Correlation analysis of SNGH18, LCAL1, IGFL2-AS1, LINC02301, and LINC01508 long non-coding RNA expression with clinical and morphologic characteristics of the disease*

Parameter	SNGH18	LCAL1	IGFL2-AS1	LINC02301	LINC01508
Age:					
r	–0,053	–0.109	–0,042	0,015	–0,169
p	0,840	0,674	0,874	0,956	0,513
Gender:					
r	0,000	0,149	0,112	0,075	–0,075
p	1,000	0,618	0,721	0,816	0,816
Stage:					
r	0,122	–0,268	–0,073	0,173	0,099
p	0,669	0,315	0,813	0,530	0,736
Tumor size:					
r	0,264	–0,132	0,184	0,160	0,267
p	0,328	0,646	0,506	0,569	0,321
Nodal status:					
r	0,000	–0,168	–0,120	0,122	0,244
p	1,000	0,541	0,673	0,669	0,363
Metastasis:					
r	–0,094	–0,063	–0,346	–0,287	–0,096
p	0,768	0,859	0,197	0,294	0,766
Grade:					
r	–0,126	0,075	–0,251	–0,204	0,178
p	0,660	0,808	0,350	0,456	0,518

Note. *r – Spearman's correlation coefficient; p – significance level.*

Table 4. *Statistical analysis of prognostic significance of long non-coding RNA SNGH18, LCAL1, IGFL2-AS1, LINC01508, LINC02301*

Indicators studied	Univariate analysis		Multivariate analysis	
	Hazard ratio	*p*	Hazard ratio	*p*
SNGH18 (high/low)	0,476	0,142	0,186	0,876
LCAL1 (high/low)	1,29	0,615	0,155	0,488
IGFL2-AS1 (high/low)	0,374	0,039*	2,454	0,692
LINC02301 (high/low)	0,682	0,462	8,346e+033	0,588
LINC01508 (high/low)	0,918	0,864	0,002	0,836

**Statistically significant.*

towards prognostic significance. In cases of high tumor infiltration by immunosuppressive phenotype macrophages, the median survival was 20.1 months, whereas in low infiltration cases, it was only 8.3 months. No patterns were found for the overall macrophage content or for cytotoxic phenotype macrophages.

Subsequently, a correlation analysis was performed to examine the relationship between the presence of different macrophage phenotypes in the tumor stroma and the expression of the investigated lncRNAs. The results of this analysis are presented in table 5.

As indicated by the results, lncRNA LINC01508 shows a significant positive correlation with both the total number of macrophages and the counts of cytotoxic and immunosuppressive phenotype macrophages. This suggests that LINC01508 may be expressed not only in tumor cells but also in macrophages; however, this hypothesis requires further investigation and verification.

Table 5. *Correlation analysis of SNGH18, LCAL1, IGFL2-AS1, LINC02301, and LINC01508 lncRNA expression with the phenotype of tumor stroma macrophages*

Parameter	SNGH18	LCAL1	IGFL2-AS1	LINC02301	LINC01508
CD68:					
r	0,328	0,053	0,479	0,095	0,579
p	0,198	0,842	0,050*	0,938	0,017*
CD163:					
r	0,225	0,186	0,445	–0.021	0,496
p	0,382	0,472	0,075	0,713	0,045*
iNOS:					
r	0,359	–0,490	0,166	0,089	0,567
p	0,176	0,037*	0,544	0,743	0,004*

**Statistically significant.*
Note. *r – Spearman's correlation coefficient; p – significance level.*

Interestingly, the expression of lncRNA LCAL1 displays an inverse correlation with the presence of cytotoxic macrophages in the tumor stroma.

DISCUSSION

This study aims to explore the clinical and prognostic significance of the lncRNAs SNGH18, LCAL1, IGFL2-AS1, LINC02301, and LINC01508, and to analyze the correlation between their expression and macrophage infiltration in the tumor stroma across different phenotypes. It is known that a large portion of the human genome is transcribed into non-coding RNAs, such as microRNAs (miRNAs), lncRNAs, small nuclear RNAs, and others [8]. On one hand, most non-coding RNAs are still poorly understood; on the other hand, some have been identified as oncogenes or tumor suppressors, and their functional mechanisms have been studied in detail [9]. lncRNAs are molecules longer than 200 nucleotides that can regulate the expression of various genes at transcriptional, post-transcriptional, and epigenetic levels [10]. Aberrant expression of lncRNAs in tumors can serve as diagnostic and prognostic markers for many oncological diseases.

Previously, we demonstrated that tumor cells of various etiologies can develop resistance to the cytotoxic activity of macrophages. Transcriptome sequencing revealed lncRNAs whose expression changed in cell lines resistant to the cytotoxic activity of macrophages [7]. According to the limited literature data, all the lncRNAs studied in this work have tumor-promoting properties, and their expression contributes to disease progression [11, 12]. It is worth noting that the expression of SNGH18, LCAL1, IGFL2-AS1, LINC02301, and LINC01508 in squamous cell carcinoma of the esophagus has not been previously studied. We were the first to show that the expression of lncRNA LCAL1 and LINC01508 in tumor tissue is higher compared to normal tissue, while the expression of lncRNA LINC02301 was found to be reduced. The expression of lncRNAs SNGH18 and IGFL2-AS1 did not significantly change. The ROC analysis conducted showed that the expression of these lncRNAs is not suitable for diagnosing squamous cell carcinoma of the esophagus.

Literature indicates that lncRNA PGM5-AS1, which acts as a tumor growth suppressor and is characterized by reduced expression in esophageal tumors, can be used for diagnosing this pathology [8]. Interestingly, in our study, we observed higher expression of LINC01508 in esophageal tumor tissue. In our previous research on non-small cell lung cancer samples, we found that the expression of this lncRNA was significantly decreased in tumor tissue compared to normal tissue, indicating that the change in expression of this molecule during tumor development is not universal and is tissue-specific [13].

In tumors of various etiologies, the expression of the lncRNAs we studied has been shown to hold clinical significance. For example, high expression of SNHG18 promotes the proliferation and invasion of non-small cell lung cancer cells [14] and is a predictor of poor prognosis in bladder cancer [15]. In our study, no association was found between the expression of lncRNAs SNGH18, LCAL1, IGFL2-AS1, LINC02301, and LINC01508 with the clinicopathological characteristics of the disease, such as tumor differentiation and stage; however, a connection was established with disease prognosis and the phenotype of macrophages infiltrating the tumor stroma. We demonstrated that the expression of lncRNA LINC01508 significantly correlates positively with the presence of macrophages of all phenotypes. This suggests that this lncRNA may be expressed not only in tumor cells but also in macrophages, although this hypothesis requires validation and further investigation. Interestingly, the expression of lncRNA LCAL1 inversely correlates with the content of cytotoxic macrophages in the tumor stroma, leading to the hypothesis that this lncRNA may play a role in shaping the phenotype of immune cells in the esophageal stroma and confirming its tumor-promoting function in this type of tumor.

Literature also presents data demonstrating a correlation between the expression of lncRNA IGFL1-AS1 and the total number of macrophages in the tumor stroma [16], which is consistent with our findings.

Literature also indicates that lncRNAs IGFL2-AS1 and SNGH18 serve as markers of poor prognosis for various types of tumors, such as colorectal cancer [16] and renal cell carcinoma [17]. Our analysis of the prognostic significance of the studied lncRNAs demonstrated that only IGFL2-AS1 is a prognostically significant factor, with its high expression associated with better survival in patients with ESCC. The median overall survival in patients with high expression of IGFL2-AS1 is twice as high compared to patients with low expression. Similar patterns were observed for lncRNA SNGH18, where the median survival for patients with high expression of this lncRNA is 20 months, while for patients with low expression, it is 12.8 months; however, these results did not reach statistical significance.

It is important to note the limitations of this study, such as the small sample size, the retrospective nature of the work, and the polymerase chain reaction analysis of lncRNA expression conducted on tumor tissue samples as a whole, without separating them into cell populations.

In summary, research on lncRNAs in esophageal cancer is still in its early stages and faces several challenges and limitations, particularly regarding clinical application. Most studies indicate that a clinically significant diagnostic tool will likely involve analyzing broad panels of markers rather than individual lncRNAs. These panels still need to be identified and validated before they can be effectively used in clinical practice.

CONCLUSION

The present study demonstrated that aberrant expression of lncRNAs LCAL1, LINC02301, and LINC01508 is observed in esophageal tumors. In contrast, lncRNA IGFL2-AS1 emerged as a significant marker of favorable prognosis for this type of tumor, despite no observed changes in its expression between normal and tumor tissues. With further fundamental and applied research, these lncRNAs may become promising markers or therapeutic targets for oncological diseases, including malignant esophageal tumors.

REFERENCES / ЛИТЕРАТУРА

1. Chen J., Liu X., Zhang Z. et al. Early diagnostic markers for esophageal squamous cell carcinoma: copy number alteration gene identification and cfDNA detection. Lab Invest 2024;104(10):102127. DOI: 10.1016/j.labinv.2024.102127
2. Wang C., Wang J., Chen Z. et al. Immunohistochemical prognostic markers of esophageal squamous cell carcinoma: a systematic review. Chin J Cancer 2017;36(1):65. DOI: 10.1186/s40880-017-0232-5
3. Ma K., Kalra A., Tsai H.L. et al. Accurate nonendoscopic detection of esophageal squamous cell carcinoma using methylated DNA biomarkers. Gastroenterology 2022;163(2):507–9. DOI: 10.1053/j.gastro.2022.04.021
4. Mohr A.M., Mott J.L. Overview of microRNA biology. Semin Liver Dis 2015;35(1):3–11. DOI: 10.1055/s-0034-1397344
5. Kovaleva O.V., Rashidova M.A., Samoilova D.V. et al. Immunosuppressive phenotype of esophagus tumors stroma. Anal Cell Pathol (Amst) 2020;2020:5424780. DOI: 10.1155/2020/5424780
6. Mantovani A., Allavena P., Marchesi F., Garlanda C. Macrophages as tools and targets in cancer therapy. Nat Rev Drug Discov 2022;21(11):799–820. DOI: 10.1038/s41573-022-00520-5
7. Kovaleva O.V., Podlesnaya P.A., Vasileva M.V. et al. Transcriptome of Lung Cancer Cells Resistant to the Cytotoxic Activity of Macrophages. Dokl Biochem Biophys 2022;507(1):312–7. DOI: 10.1134/S160767292205009X
8. Zhou M., Bao S., Gong T. et al. The transcriptional landscape and diagnostic potential of long non-coding RNAs in esophageal squamous cell carcinoma. Nat Commun 2023;14(1):3799. DOI: 10.1038/s41467-023-39530-1
9. Han Y., Zhao G., Shi X et al. The emerging role of long non-coding rnas in esophageal cancer: functions in tumorigenesis and clinical implications. Front Pharmacol 2022;13:885075. DOI: 10.3389/fphar.2022.885075
10. Подлесная П.А., Ковалева О.В., Петренко А.А. и др. Новые длинные некодирующие РНК в онкогенезе рака легкого. Молекулярная медицина 2023;21(5):3–11. DOI: 10.29296/24999490-2023-05-01
Podlesnaya P.A., Kovaleva O.V., Petrenko A.A. et al. New long non-coding RNAs in lung cancer tumorigenesis. Molekulyarnaya meditsina = Molecular Medicine 2023;21(5):3–11. (In Russ.). DOI: 10.29296/24999490-2023-05-01
11. Ouyang D., Su J., Huang P. et al. Identification of lncRNAs via microarray analysis for predicting HER2-negative breast cancer response to neoadjuvant chemotherapy. Int J Clin Expe Pathol 2018;11(5):2621–8.
12. Wu Z., Ouyang C., Peng L. Risk assessment model and nomogram established by differentially expressed lncRNAs for early-stage lung squamous cell carcinoma. Transl Cancer Res 2020;9(9):5304–14. DOI: 10.21037/tcr-20-999
13. Ковалева О.В., Подлесная П.А., Мочальникова В.В. и др. Клиническая значимость экспрессии днРНК LINC01508 при немелкоклеточном раке легкого. Бюллетень экспериментальной биологии и медицины 2024;177(4):502–5. DOI: 10.47056/0365-9615-2024-177-4-502-505
Kovaleva O.V., Podlesnaya P.A., Mochalnikova V.V. et al. Clinical significance of LINC01508 lncRNA expression in non-small cell lung cancer. Byulleten eksperimentalnoy biologii i meditsiny = Bulletin of Experimental Biology and Medicine 2024;177(4):502–5. (In Russ.). DOI: 10.47056/0365-9615-2024-177-4-502-505
14. Fan H., Yuan J., Li Y. et al. MKL1-induced lncRNA SNHG18 drives the growth and metastasis of non-small cell lung cancer via the miR-211-5p/BRD4 axis. Cell Death Dis 2021;12(1):128. DOI: 10.1038/s41419-021-03399-z
15. Ochoa A., Zhang J., Choi W. et al. MP72-06 The long non-coding RNA snhg18 promotes PPARγ function and "luminal" gene expression in muscle-invasive bladder cancer. J Urology 2015;193(4S):e923. DOI: 10.1016/j.juro.2015.02.2640
16. Cen X., Huang Y., Lu Z. et al. LncRNA IGFL2-AS1 promotes the proliferation, migration, and invasion of colon cancer cells and is associated with patient prognosis. Cancer Manag Res 2021;13:5957–68. DOI: 10.2147/CMAR.S313775
17. Pan Y., Lu X., Shu G. et al. Extracellular vesicle-mediated transfer of lncRNA IGFL2-AS1 confers sunitinib resistance in renal cell carcinoma. Cancer Res 2023;83(1):103–16. DOI: 10.1158/0008-5472.CAN-21-3432

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