Volume 14, Issue 1, Page 48-57, 2025 https://doi.org/10.46810/tdfd.1585199 Research Article

BİNGÖL UNIVERSITY

Türk Doğa ve Fen Dergisi
Turkish Journal of Nature and Science
www.dergipark.gov.tr/tdfd

The Potential Prognostic Value of Glutatione-S Transferase Izoenzymes in Non-small Cell Lung Cancer

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(Received: 14.11.2024, Accepted: 14.12.2024, Online Publication: 26.03.2025)

Keywords
Lung Cancer, Glutatione-S Transferase, Caspase-3, Bcl-2, p38, p53

Abstract: To investigate correlations between GST isozyme levels and tumor markers to evaluate the prognostic value of GST isozymes. This retrospective study analyzed clinical data from 40 patients with adenocarcinoma and squamous cell carcinoma. Tumor and adjacent healthy tissue samples were immunohistochemically stained to profile GST enzymes (Sigma, Omega, Pi, Mu) and caspase-3, Bcl-2, p38, p53. Associations between protein expression levels and patient characteristics were examined, and correlations between GST enzymes and Caspase-3, Bcl-2, p38, p53 were analyzed. Significant immunohistochemical differences were found between tumorous and healthy tissues for all markers. GST enzymes (GSTS, GSTO, GSTP, GSTM) were predominantly expressed in tumorous tissues, with GSTO and GSTP showing high expression levels. Compared to SCC tissues, GSTP expression is around 30% higher in AC tissues. In contrast, GSTO expression increases by around 25% in second-stage tumors, particularly in AC tissues. Correlation analysis revealed significant positive associations between Bcl-2 and caspase-3, p38, GSTS, between caspase-3 and GSTP, and between p38 and GSTM in tumor tissues. The study supports the prognostic value of GST isozymes in NSCLC.

Küçük Hücreli Dışı Akciğer Kanserinde Glutatione-S Transferaz İzoenzimlerinin Potansiyel Prognostik Değeri

Anahtar Kelimeler
Akciğer Kanseri,
Glutatyon-S Transferaz,
Kaspaz-3,
Bcl-2,
p38,
p53

Öz: GST izozimlerinin prognostik değerini değerlendirmek için GST izozim düzeyleri ile tümör belirteçleri arasındaki korelasyonları araştırmak. Bu retrospektif çalışmada adenokarsinom ve skuamöz hücreli karsinomu olan 40 hastanın klinik verileri analiz edilmiştir. Tümör ve komşu sağlıklı doku örnekleri, GST enzimlerinin (Sigma, Omega, Pi, Mu) ve kaspaz-3, Bcl-2, p38, p53'ün profilini çıkarmak için immünohistokimyasal olarak boyandı. Protein ekspresyon düzeyleri ile hasta özellikleri arasındaki ilişkiler incelenmiş ve GST enzimleri ile Kaspaz-3, Bcl-2, p38, p53 arasındaki korelasyonlar analiz edilmiştir. Tüm belirteçler için tümörlü ve sağlıklı dokular arasında anlamlı immünohistokimyasal farklılıklar bulunmuştur. GST enzimleri (GSTS, GSTO, GSTP, GSTM) ağırlıklı olarak tümörlü dokularda eksprese edilmiş, GSTO ve GSTP yüksek ekspresyon seviyeleri göstermiştir. SCC dokularıyla karşılaştırıldığında, GSTP ekspresyonu AC dokularında yaklaşık %30 daha yüksektir. Buna karşılık, GSTO ekspresyonu ikinci evre tümörlerde, özellikle de AC dokularında yaklaşık %25 oranında artmaktadır. Korelasyon analizi, tümör dokularında Bcl-2 ile kaspaz-3, p38, GSTS arasında, kaspaz-3 ile GSTP arasında ve p38 ile GSTM arasında anlamlı pozitif ilişkiler olduğunu ortaya koymuştur. Çalışma, KHDAK'de GST izozimlerinin prognostik değerini desteklemektedir.

1. INTRODUCTION

Tumors arising in the lung parenchyma or within the bronchi are referred to as lung cancer, or bronchogenic carcinoma. Lung neoplasms are the primary cause of cancer incidence and death globally [1].

Many of the more advanced methods of pathologic diagnosis have resulted in a more accurate pathologic and genetic categorization of lung tumors, opening the door to more effective therapeutic options. This has been made possible by the introduction of immunohistochemistry and molecular testing throughout the classification. The 2021 WHO Classification of Thoracic Tumors includes following main titles:: papillomas, adenomas, precursor glandular lesions, adenocarcinoma in situ, adenocarcinomas (AC), invasive nonmucinous adenocarcinoma, squamous precursor lesions, squamous cell carcinomas (SCC), large cell carcinomas (LCC), adenosquamous carcinomas, sarcomatoid carcinomas, salivary gland-type tumors, neuroendocrine tumors, neuroendocrine carcinomas (e.g., small cell carcinoma, SCLC, and large cell neuroendocrine carcinoma, LCNEC), tumors of ectopic tissues (malonoma and meningioma), mesenchymal tumors specific to the lung, and PEComatous tumors [2]. AC, SCC, and LCC are subtypes of non-small-cell lung carcinoma (NSCLC), which makes up 85% of all cases of lung cancer [3].

The pathology of adenocarcinomas is characterised by the formation of neoplastic glands, the expression of pneumocyte marker (thyroid transcription factor 1 (TTF-1) with or without napsin expression), or intracytoplasmic mucin. On cytology, squamous cell pathology is indicated by the presence of keratin and/or intercellular desmosomes [4]. Both of them requires the immunohistochemical (IHC) evidence of expression of some current markers such as p38 [5,6] caspase-3[7-9], Bcl-2 [10-11] and p53 [12-14]. However, despite the fact that these markers are widely employed, some publications have critical views on their prognostic efficacy as well as negative attitudes [15,16]. The key takeaway is that there is a constant mention of the need for additional IHC markers that can serve as substitutes for these and other related popular indicators, or that, when combined, can improve prognostic outcomes.

A multiple gene family of phase II enzymes known as glutathione transferases (GSTs) catalyses endogenous glutathione detoxification processes and shields cellular macromolecules from cytotoxic and carcinogenic chemicals. The six gene families that make up the cytosolic GST isozymes are alpha (GSTA), mu (GSTM), theta (GSTT), pi (GSTP), omega (GSTMO), and one membrane-associated microsomal GST, which are categorised based on their biochemical properties [17]. Abnormal expression and GST polymorphisms have been linked to a number of human diseases, including malignant tumors. Many tumors have increased GST expression, which is linked to poor prognosis, treatment resistance, and cell proliferation [18]. There are few studies on the prognostic value of GST isozymes in lung cancer, despite the fact that many cancer types, including myeloid leukaemia, stomach, urinary bladder, breast, and nasopharyngeal carcinoma, have had this information evaluated and reported [19-23]. However, it is still possible to modify the risk of lung cancer by changing the structure, function, or expression levels of specific isozymes [24].

In the current study, the expressions of p38, p53, Bcl-2 and caspase-3, which are generally accepted cancer markers, as well as GSTO1, GSTM1, GSTP1 and GSTS1 isozymes were detected in two different types of NSCLC tumor samples, AC and SCC. The expression levels of those proteins in cancerous tissues were comparatively examined by using peripheral tissue as control group for each patient and the prognostic values of GST isozymes were analyzed by cross comparisons.

2. MATERIAL AND METHOD

2.1. The sources of specimens

The Kartal Dr. Lütfi Kırdar City Hospital Pathology Clinic in Istanbul, Turkey, identified and treated 40 patients with 20 AC and 20 SCC for the study. The clinical information and state of follow-up were reviewed in the patient's medical file. The median duration of follow-up was 24 months (between 2017-2019). Clinical staging, adequate follow-up information, and slides showing the histology of the tumor were available to all patients. Each patient was staged at the time of surgery using regional lung tissue dissection. The patient's cancer stage was ascertained using the TNM staging method created by the American Joint Committee on Cancer. Of the 40 cases, 29 were men and 11 were women. The mean age of the patients was 67.20±1.36 years, with 62.5% of them being over 65. Of the 40 lung tumors that underwent surgical removal, 13 were in stage 1A, 7 in stage 1B, 8 in stage 2A, 9 in stage 2B, and 3 in stage 3A. 3.67±0.38 cm is the average tumor diameter. Table 1 summarises the following information: patient age and gender; cancer grade; localization, invasion, involvement, in situ, metastasis, and neoadjuvant states; tumor size and stage; and patient survival states.

Table 1. Demographic and clinical data of patients

Data	Category	n (%)
Demographic data		
Gender	Female	11 (27.5%)
	Male	29 (72.5%)
Age	≤65	15 (37.5%)
	>65	25 (62.5%)
Clinical data		
Diagnosis	AC	20 (50%)
	SCC	20 (50%)
Grade	Acinar	5 (12.5%)
	Keratinized	10 (25.0%)
	Lepidic	5 (12.5%)
	Non-keratinized	10 (25.0%)
	Papiller	5 (12.5%)
	Solid	5 (12.5%)
Localization	Right AC	1 (2.5%)
	Right lower lobe	11 (27.5%)
	Right upper lobe	12 (30.0%)
	Left AC	2 (5.0%)
	Left lower lob	2 (5.0%)
	Left upper lob	11 (27.5%)
	Upper lobe	1 (2.5%)
Vascular invasion	Yes	13 (32.5%)
	No	27 (67.5%)
Neural invasion	Yes	9 (22.5%)
	No	31 (77.5%)
Bronchial involvement	Yes	11 (27.5%)
	No	29 (72.5%)
Pleural involvement	Yes	12 (30.0%)
	No	28 (70.0%)
in situ	Yes	4 (10.0%)
	No	36 (90.0%)
Metastasis	Yes	13 (32.5%)
	No	27 (67.5%)
Tumor size	T1A	11 (27.5%)
	T1B	5 (12.5%)
	T2A	12 (30.0%)
	T2B	4 (10.0%)
	T3	8 (20.0%)
Lymph node metastasis	N0	29 (72.5%)
	N1	7 (17.5%)
	N2	3 (7.5%)
	No data	1 (2.5%)
Stage	1A	13 (32.5%)
	1B	7 (17.5%)
	2A	8 (20.0%)
	2B	9 (22.5%)
	3A	3 (7.5%)
Neoadjuvant	Yes	2 (5.0%)
	No	38 (95.0%)
Survival status	Dead	9 (22.5%)
	Alive	31 (77.5%)
n % AC SCC	number of patients number of patients in the specified group / total number of patients adenocarcinoma squamous cell carcinoma	

2.2. Immunohistochemical analyses

The specimens that were surgically removed were routinely embedded in paraffin blocks and preserved in 10% formalin. After being sliced at 4 µm, tissue sections were stained with hematoxylin and eosin. Endogenous peroxidase activity was inhibited for immunohistochemistry by soaking the sections in 1% hydrogen peroxide (v / v) in methanol for ten minutes at room temperature (RT). Following that, the sections were rinsed with distilled water for five minutes. A household pressure cooker was used to accomplish antigen retrieval for three minutes using 0.01 M citrate buffer (pH 6.0). The sections were treated with super block (SHP125) (ScyTek Laboratories, USA) at room temperature for 10 minutes in order to prevent nonspecific background staining. The sections were incubated with diluted primary antibody anti-bcl-2 (Boster, USA, dilution 1:250), Anti-caspase-3 (Genex, USA, dilution 1:500), Anti-p38 (Santa Cruz, USA, dilution 1:500), Anti-p53 (Boster, USA, dilution 1:200) and Anti-GSTP (Boster, USA, dilution 1:1000), Anti-GSTM (Santa Cruz, USA, dilution 1:500), Anti-GSTO (Abcam, USA, dilution 1:200), Anti-GSTS (Santa Cruz, USA, dilution 1:50), for 1 hour at room temperature (RT). The sections were incubated at RT for biotinylated link antibody (SHP125) (ScyTek Laboratories, USA) following a 15-minute washing in TBS. Streptavidin/HRP complex (SHP125) (ScyTek Laboratories, USA) was then used as a course of treatment. To observe peroxidase activity in the tissues, diaminobenzidine (DAB) was utilised. Following a brief hematoxylin counterstain, the sections were dried and mounted. The degree of immunostaining of tumor cells was assessed as follows: The intensity of the cytoplasmic staining is 1 for weak, 2 for moderate, and 3 for strong. The intensity of immunostaining for GST Pi, GST Mu, GST Sigma, GST Omega, caspase 3, p38, Bcl-2 were assessed using the following scale: (Figure 1A-G) 1 = weak, 2 = moderate, 3 = strong. Immunohistochemical p53 reactivity was evaluated as follows: (null) for negative staining (no protein expression), (wild) for weak staining, (mutant) for strong staining (Figure 1H).

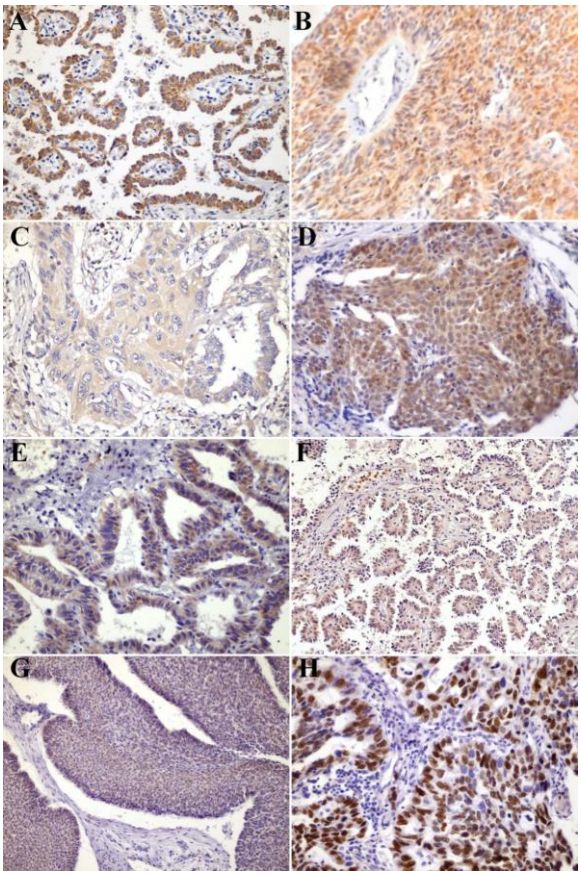

Figure 1. Composite figures of immunohistochemical staining results of (A) GST Pi, (B) GST Mu, (C) GST Sigma, (D) GST Omega (E) caspase 3, (F) p38, (G) Bcl-2, (H) p53

2.3. Statistical analyses

IBM® SPSS® Statistics 25.0 was used to conduct statistical analyses. Our investigation included normal tissues and SCC and AC tumors from 40 patients. The findings are given as mean +/- standard error of mean (SEM). Data that has been categorised according to clinical and demographic traits is presented as percentages and numbers. The Shapiro-Wilk test was used to assess the data's distribution patterns. Using the Levene test, homogeneity of variances was investigated. The Mann-Whitney U test was used to compare pairs when the assumptions of the parametric test were not met. The Chi-square test was used to investigate the association between category variables. Correlation analyses were conducted using Spearman's rank correlation test. The accepted threshold for statistical significance was $p<0.05$.

3. RESULTS

Bcl-2, Caspase-3, p38 expressions in tumor and normal tissues were examined immunohistochemically and expression levels were evaluated (Table 3). Weak Bcl-2 expression was found in 12.5% of tumor tissues and moderate Bcl-2 expression was found in 7.5%. Bcl-2 expression was not detected in any of the normal tissues. Bcl-2 expression of tumor tissues is statistically significantly higher than that of normal tissues ($p = 0.003$). Caspase-3 was weakly expressed in 20% of tumor tissues and moderately expressed in 2.5%. Caspase-3 expression was not found in normal tissues. Caspase-3 expression in tumor tissues is statistically significantly higher than in normal tissues ($p = 0.002$). p38 was expressed weakly in 52.5% of tumor tissues, moderately in 25%, and strongly in 10%. While weak p38 expression is seen in 7.5% of normal tissues, there are no tissues where it is expressed at moderate or strong levels. The p53 expression of tumor tissues is statistically significantly higher than that of normal tissues ($p<0.001$). The p53 staining patterns of tumor tissues were evaluated and described as mutant (more than 40% of the positive cells), null (less than 40%of the positive cells) and wild (40% of the positive cells).

Table 2. Bcl-2, caspase-3, p38, p53 expression levels of tumor and normal tissues determined by immunohistochemistry

IHC Score	Bcl-2		Caspase-3		p38		p53	
	Tumor	**Normal**	**Tumor**	**Normal**	**Tumor**	**Normal**	**Staining pattern**	**Tumor**
0	32/40 (%80.0)[a]	40/40 (%100)[a]	31/40 (%77.5)[a]	40/40 (%100)[a]	5/40 (%12.5)[a]	37/40 (%92.5)[a]	Mutant	12/40 (%30.0)[a]
1	5/40 (%12.5)[a]	-	8/40 (%20.0)[a]	-	21/40 (%52.5)[a]	3/40 (%7.5)[a]	Null	20/40 (%50.0)[a]
2	3/40 (%7.5)[a]	-	1/40 (%2.5)[a]	-	10/40 (%25.0)[a]	-	Wild	8/40 (%20.0)[a]
3	-	-	-	-	4/40 (%10.0)[a]	-		
Avg.	0.28 ± 0.09[b*] (0-2)[c]	-	0.25 ± 0.08[b*] (0-2)[c]	-	1.33 ± 0.13[b*] (0-3)[c]	0.08 ± 0.04[b] (0-1)[c]		
p-value	***0.003***		***0.002***		***<0.001***			
T/P value	-		-		*16.63*			

Total n = 40

Scoring was made according to the staining intensity of the tissues. 0: no staining, 1: weak positive, 2: moderate positive, 3: strong positive

(*) $p<0.05$ is statistically significant according to the Mann-Whitney U test

([a]) number of samples stained at the specified score / total number of samples

([b]) Mean ± SEM

([c]) min – max

GSTS, GSTO, GSTP and GSTM expressions of tumor and normal tissues were evaluated immunohistochemically (Table 3). While weak and moderate GSTS expression was found in 70% of tumor tissues, weak and moderate GSTS expression was observed in 42.5% of normal tissues. Although the T/P ratio was 1.35, there was no significant difference between the GSTS expression levels of tumor and normal tissues ($p = 0.051$). GSTO was weakly expressed in 55% of tumor tissues and moderately expressed in 37.5%.

While medium and strong levels of GST-O expression were not observed in normal tissues, weak expression was observed in only 1 tissue. GSTO expressions of tumor tissues were found to be significantly higher than that of normal tissues ($p<0.001$). It was observed that GSTP was expressed weakly in 37.5% of tumor tissues, moderately in 17.5%, and strongly in 37.5%. While strong GSTP expression was not observed in normal tissues, weak expression was detected in 7.5% of the tissues and moderate expression was detected in 5% of the tissues. GSTP showed significantly higher expression in tumor tissues than in normal tissues ($p<0.001$). While GSTM was expressed weakly in 10% of tumor tissues, moderately in 7.5%, and strongly in 5.2%, it was observed that it was not expressed at all in normal tissues. The expression of GSTM in tumor tissues was found to be significantly higher than in normal tissues ($p = 0.003$).

Table 3. Immunohistochemically detected GSTS, GSTO, GSTP and GSTM expression levels of tumor and normal tissues

IHC Score	GSTS		GSTO		GSTP		GSTM	
	Tumor	**Normal**	**Tumor**	**Normal**	**Tumor**	**Normal**	**Tumor**	**Normal**
0	5/40 (%12.5)[a]	16/40 (%40.0)[a]	3/40 (%7.5)[a]	38/40 (%95.0)[a]	3/40 (%7.5)[a]	35/40 (%87.5)[a]	32/40 (%80.0)[a]	40/40 (%100)[a]
1	28/40 (%70.0)[a]	17/40 (%42.5)[a]	22/40 (%55.0)[a]	1/40 (%2.5)[a]	15/40 (%37.5)[a]	3/40 (%7.5)[a]	4/40 (%10.0)[a]	-
2	7/40 (%17.5)[a]	7/40 (%17.5)[a]	15/40 (%37.5)[a]	-	7/40 (%17.5)[a]	2/40 (%5.0)[a]	3/40 (%7.5)[a]	-
3	-	-	-	-	15/40 (%37.5)[a]	-	1/40 (%2.5)[a]	-
Avg.	1.05 ± 0.09 (0-2)[c]	0.78 ± 0.12 (0-2)[c]	1.30 ± 0.10* (0-2)[c]	0.03 ± 0.03 (0-1)[c]	1.85 ± 0.16* (0-3)[c]	0.18 ± 0.08 (0-2)[c]	0.33 ± 0.12* (0-3)[c]	-
p-value	**0.051**		**<0.001**		**<0.001**		**0.003**	
T/P value	1.35		43.33		10.28		-	

Total n = 40

Scoring was made according to the staining intensity of the tissues. 0: no staining, 1: weak positive, 2: moderate positive, 3: strong positive.

(*) $p<0.05$ is statistically significant according to the Mann-Whitney U test

([a]) number of samples stained at the specified score / total number of samples

([b]) Mean ± SEM

([c]) min – max

Correlation analyzes were performed between Bcl-2, Caspase-3, p38, GSTS, GSTO, GSTP, GSTM expressions of tumor and normal tissues; and the results are stated in Table 4.

Table 4. Correlation analyzes of IHC expressions in tumor and normal tissues

		Bcl-2	Caspase-3	p38	p38	GSTS	GSTS	GSTO	GSTO	GSTP	GSTP	GSTM
Bcl-2 Tumor	r	1.000	**0.468****	**0.387***	**0.342***	**0.402***	-0.031	0.043	-0.082	0.137	-0.023	0.272
	p		**0.002**	**0.014**	**0.031**	**0.010**	0.850	0.793	0.620	0.399	0.889	0.089
Caspase-3 Tumor	r	**0.468****	1.000	0.223	0.289	0.058	0.177	0.001	-0.082	**0.451****	-0.203	0.184
	p	**0.002**		0.166	0.071	0.722	0.273	0.997	0.619	**0.003**	0.210	0.254
p38 Tumor	r	**0.387***	0.223	1.000	**0.359***	0.307	-0.031	0.058	-0.071	0.046	-0.161	**0.374***
	p	**0.014**	0.166		**0.023**	0.054	0.848	0.724	0.670	0.779	0.322	**0.017**
p38 Normal	r	**0.342***	0.289	**0.359***	1.000	0.148	0.080	0.005	-0.047	-0.039	-0.107	0.136
	p	**0.031**	0.071	**0.023**		0.363	0.623	0.977	0.777	0.810	0.510	0.404
GSTS Tumor	r	**0.402***	0.058	0.307	0.148	1.000	-0.101	0.216	-0.027	0.197	0.120	0.090
	p	**0.010**	0.722	0.054	0.363		0.534	0.181	0.869	0.224	0.462	0.582
GSTS Normal	r	-0.031	0.177	-0.031	0.080	-0.101	1.000	-0.147	-0.180	0.011	0.240	-0.117
	p	0.850	0.273	0.848	0.623	0.534		0.366	0.272	0.946	0.136	0.474
GSTO Tumor	r	0.043	0.001	0.058	0.005	0.216	-0.147	1.000	0.195	0.228	0.097	-0.070
	p	0.793	0.997	0.724	0.977	0.181	0.366		0.234	0.157	0.550	0.669
GSTO Normal	r	-0.082	-0.082	-0.071	-0.047	-0.027	-0.180	0.195	1.000	0.191	-0.062	-0.075
	p	0.620	0.619	0.670	0.777	0.869	0.272	0.234		0.244	0.707	0.648
GSTP Tumor	r	0.137	**0.451****	0.046	-0.039	0.197	0.011	0.228	0.191	1.000	-0.030	0.281
	p	0.399	**0.003**	0.779	0.810	0.224	0.946	0.157	0.244		0.854	0.079
GSTP Normal	r	-0.023	-0.203	-0.161	-0.107	0.120	0.240	0.097	-0.062	-0.030	1.000	-0.187
	p	0.889	0.210	0.322	0.510	0.462	0.136	0.550	0.707	0.854		0.247
GSTM Tumor	r	0.272	0.184	**0.374***	0.136	0.090	-0.117	-0.070	-0.075	0.281	-0.187	1.000
	p	0.089	0.254	**0.017**	0.404	0.582	0.474	0.669	0.648	0.079	0.247	

r	correlation coefficient
p	limit of significance (sign. (2-tailed))
(*)	The correlation was significant at the 0.05 level (2-tailed).
(**)	The correlation was significant at the 0.01 level (2-tailed).

Positive and significant correlations were found between Bcl-2 and caspase-3, p38 and GSTS expressions in tumor tissues ($p<0.05$). A positive and significant correlation was determined between caspase-3 and GSTP expressions in tumor tissues ($r=0.451$, $p=0.003$). A positive and significant correlation was found between p38 and GSTM expressions in tumor tissues ($r=0.374$, $p=0.017$). A positive and significant correlation was also observed between p38 expressions of tumor and normal tissues ($r = 0.359$, $p = 0.023$).

4. DISCUSSION

The global epidemiology of lung cancer need ongoing surveillance due to its exceptional disease burden and the regional variations in trends for ageing, smoking, and population growth. When categorizing lung malignancies for treatment and preventive measures, the histology and molecular markers of the disease are crucial factors.

GST isozymes have attracted the interest of cancer researchers because they are expressed in all cell types and are abundant in aggressive cancer cells, implying that they play an important role in tumor growth and pathogenicity. The expression levels of several common cancer markers and GSTs, separately in AC and SCC, were assessed in the current study in connection with certain patient demographic information, and some conclusions were drawn. Regardless of the type of lung cancer, it has been noted that the expression of the GSTP and GSTO isozymes differs in male and female patients. Specifically, the expression of the GSTP isozyme was found to be approximately 11% greater in female patients, whilst the GSTO isozyme was 13% higher in male patients. Gender-related expression differences have also been demonstrated in other studies; e.g. according to Pan et al.,[25] lung cancer patients' null genotypes of GSTT and GSTM were associated with a gender-related risk [25].

Comparing the expressions of GSTO and GSTP isozymes for SCC and AC tumors, it was shown that, while taking into account the values obtained in AC tissues, GSTO was 7.7% higher in SCC tissue samples. Conversely, the GSTP isozyme exhibited the most intriguing data, with a 32.4% increase in expression in AC tissues relative to SCC. Wang et al. [26] noted the upregulation of GSTP in their recent study using lung cancer organoids and reported that it might be a significant target protein, particularly in chemotherapy trials [26]. Human lung squamous-cell carcinoma resistance to chemotherapeutic drugs is associated with an overexpression of GSTP[27]. This significant enzyme may have a role in both the diagnosis and treatment planning of lung cancer, according to the results of the current study. It should be highlighted, nonetheless, that not all cancer types would support this kind of inference, and that it might differ in the instance of lung cancer based on additional factors such the cancer's stage.

In fact, our investigation revealed that variations in the GSTP isozyme were observed in both AC and SCC first- and second-stage malignant tissues (1A and 1B and 2A and 2B, respectively). While GSTP expression was shown to be roughly 7% greater in second stage sample tissues compared to first stage tissues in SCC tumors, it was found to be approximately 9% higher in first stage samples in AC tissues than in second stage tissues. Rybárová et al.[28] reported a similar outcome for non-small cell lung cancer. In cancer samples, they discovered a statistically significant connection between GSTP1 and histological grade ($p = 0.025$); also, the adenocarcinoma samples exhibited the greatest GSTP1 expression (77%) based on histopathological type [28]. Although there is limited evidence for GSTP's predictive power, there is data supporting its use as a prognostic biomarker overall [29].

A correlation between the levels of GSTO expression and the histopathological grade was found in the present study. GSTO expression in second-stage tissues was 16.1% greater than in first-grade tissues, regardless of the type of NSCLC. More significantly, the GSTO expression level found in second-grade SCC tissues is roughly 24% greater than in first-grade tissues when classified based on cancer types. These findings suggest that GSTO protein levels, similar to GSTP, have the potential to be employed as a marker in non-small cell lung cancer diagnosis and stage assessment. When compared to control tissues, there was a statistically significant increase in GSTO expression in tumor tissues. It is therefore a target for medicinal uses. GSTO1 controls the JAK/STAT3 signalling pathway, which may play a significant part in NSCLC. As a result, blocking GSTO1 expression levels could be a cutting-edge treatment approach for NSCLC [30].

In NSCLC targeted cancer therapy, p38 is a protein that is regularly investigated for its functions in malignant cell proliferation or transformation. p38 is a protein frequently studied in NSCLC targeted cancer therapy for its roles in malignant cell growth or transformation [5,31,32]and p38 inhibition can be a potent therapeutic strategy against NSCLC [33,34]. We discovered that the expression of this significant marker protein was 11.3% higher in AC type cancer tissues than in SCC tissues. Furthermore, upon reexamining the expression in these AC tissues for the first and second stages, we observed a 25.5% higher rate of p38 protein detection in first-grade tissues relative to second-grade tissues. A comparable examination for SCC was performed again, and in second-grade tissues, p38 was found to be about 9% higher. It has, also, been shown that the mutant form of p53, another important tumor suppressor gene like p38, is more frequent in AC tissues than in SCC.

Evidence in this study reveals a significant elevation of Bcl-2, caspase-3, and p38 expression in tumor tissue. Moreover, the mutant allele of p53 was associated with lower expression of p53 in tumor tissue, with a higher frequency in men. These results emphasize that these proteins, known as biomarkers in cancer pathology, may also have prognostic value in lung cancer. Research indicates that patients with non-small cell lung cancer (NSCLC) who exhibit positive expression of Bcl-2 tend to experience a more favorable prognosis compared to

those with negative Bcl-2 expression [35,36]. However, the influence of Bcl-2 expression on the survival outcomes of stage I NSCLC patients appears to be less pronounced. While Bcl-2 expression shows promise as a prognostic biomarker in lung cancer, especially in NSCLC, its precise prognostic value across various stages and types of lung cancer requires validation through further large-scale clinical trials.

Studies have shown that high caspase-3 expression is associated with apoptosis of tumor cells and may lead to a better prognosis in patients with NSCLC [37,38]. While caspase-3 is a key player in apoptotic cell death, its expression levels in lung cancer have been linked to patient outcomes, highlighting its potential as a prognostic marker in the management of NSCLC. Research indicates that p38 MAPK, particularly the isoform p38α, shows elevated expression in lung cancer tissues, with significant associations with tumor stage and prognosis [39]. Additionally, the prognostic value of p38 in lung cancer has been highlighted, with activated p38 showing increased levels in tumors compared to normal tissues, suggesting its relevance as a prognostic marker in NSCLC [40]. Patients with lung cancer who exhibit p53 mutations or abnormal p53 expression generally experience a poorer prognosis and may also demonstrate increased resistance to chemotherapy and radiation therapy [42,43]. Since p53 is a tumor suppressor gene that regulates cell division and apoptosis, its lower expression due to mutations could contribute to unchecked cell proliferation and tumor progression. Additionally, the observation of higher frequency in men could suggest gender-specific differences in the genetic and molecular characteristics of the tumor.

Bcl-2 was positively correlated with GSTS expression, while caspase-3 and p38 showed a positive correlation with GSTP and GSTM, respectively. There is a solid body of opinion about the connection between p38 and GSTM proteins. Studies suggest that GSTM proteins, particularly GSTM1, play a role in modulating stress-activated signals and the p38 signaling pathway by interacting with key proteins like Ask1. Heat shock-induced dissociation of GSTM1 from Ask1 can affect the activation of the p38 pathway. This indicates a functional relationship between p38 and GSTM proteins in cellular signaling pathways [43-45].

This study highlights several clinical features of cancer dynamics at the molecular level for the first time. Evidence from research results revealed caspase-3 was expressed higher in AC patients and among acinar tumor tissues. GSTS was significantly higher in vascular invasion and metastasis, while in pleural invasion GSTP was higher. The available information suggests that caspase-3 expression is associated with tumorigenesis and prognosis in certain cancers, but does not allow for a direct comparison between AC and SCC [7,38]. While the search results support the idea that caspase-3 expression is elevated in acinar tumors compared to normal tissues [46]. They do not directly justify the claim that caspase-3 expression is notably higher in acinar tumor tissues compared to keratinized and non-keratinized SCC tissues. To determine these specific relationships, further research focusing on this aspect would be necessary.

In a lung adenocarcinoma study performed with these markers on tissues surgically obtained from 166 patients, the significance of the protein level expression of Caspase-3, p53 and Bcl-2 revealed the effectiveness of these markers involved in the apoptosis pathway. This suggests that a correlation can be established with regard to positive contributions to prognosis, particularly in the context of lung cancers [12].

In a separate study by Oğuztüzün et al., immunohistochemical staining characteristics of glutathione-S-transferase alpha, pi, mu, theta, and p53 were investigated in 50 patients with primary lung carcinoma. A comparison of normal and tumor tissues from these cases revealed that glutathione-S-transferase alpha, pi, mu, theta expressions were significantly higher in tumor cells than in normal cells, while p53 expression did not differ significantly between the two tissue types[47].

4. CONCLUSION

Lung cancer is the most common cancer to be diagnosed and the leading cause of cancer-related deaths globally, with an estimated 2.20 million new cases and 1.79 million deaths annually [48]. Although some encouraging molecular markers have been found, there isn't a single marker that can accurately predict a patient's prognosis or response to treatment for those who suffer this terrible disease. With a better understanding of the molecular biology of lung cancer, more tailored therapies may be developed. Age, stage, and performance status are examples of traditional prognostic indicators that are still unquestionably useful; nevertheless, the discovery of additional markers may serve to further improve this and direct treatment decisions [49].

The current study's findings may point to the utility of two GST isozymes, GSTO and GSTP, in the diagnosis and treatment of non-small cell lung cancer. Compared to SCC tissues, GSTP expression is around 30% higher in AC tissues. In contrast, GSTO expression increases by around 25% in second stage tumors, particularly in AC tissues.

The study incorporates a wide array of established cancer associcated proteins p38, p53, Bcl-2, and caspase-3 along with a novel focus on glutathione transferases (GSTs), offering a comprehensive understanding of molecular markers in lung cancer pathology. The study explores the association between marker expression levels and various clinical parameters, providing insights into potential prognostic indicators and therapeutic targets in lung cancer. While the study examines the expression levels of various markers, it does not directly assess their prognostic significance or impact on patient outcomes over time, limiting the interpretation of their clinical relevance. The study is conducted at a single pathology clinic, which may limit the diversity of patient populations and tumor characteristics, potentially

influencing the extrapolation of findings to broader populations. Although the study identifies correlations between marker expression levels, it does not delve into the functional implications of these associations or mechanistic insights into cancer pathogenesis, warranting further investigation.

REFERENCES

[1] Thandra KC, Barsouk A, Saginala K, Aluru JS, & Barsouk A. (Epidemiology of lung cancer. Contemporary Oncology. 2021; *25*(1): 45. https://doi.org/10.5114/WO.2021.103829

[2] Nicholson AG, Tsao MS, Beasley MB, Borczuk AC, Brambilla E, Cooper WA, et al. The 2021 WHO Classification of Lung Tumors: Impact of Advances Since 2015. Journal of Thoracic Oncology : Official Publication of the International Association for the Study of Lung Cancer. 2022; 17(3): 362–387. https://doi.org/10.1016/J.JTHO.2021.11.003

[3] Zappa C, & Mousa SA. Non-small cell lung cancer: current treatment and future advances. Translational Lung Cancer Research. 2016; *5*(3): 288. https://doi.org/10.21037/TLCR.2016.06.07

[4] Siddiqui F, Vaqar S, Siddiqui AH . Lung Cancer. Cambridge Handbook of Psychology, Health and Medicine, Second Edition, 605–606, 2023. https://doi.org/10.1017/CBO9780511543579.138

[5] Greenberg AK, Basu S, Hu J, Yie TA, Kam MTW, Rom WN, Lee TC. Selective p38 activation in human non-small cell lung cancer. American Journal of Respiratory Cell and Molecular Biology. 2002;26(5): 558–564. https://doi.org/10.1165/AJRCMB.26.5.4689

[6] Kato S, Yokoyama S, Hayakawa Y, Li L, Iwakami Y, Sakurai H, Saiki I. P38 pathway as a key downstream signal of connective tissue growth factor to regulate metastatic potential in non-small-cell lung cancer. Cancer Science, 2016;107(10): 1416–1421. https://doi.org/10.1111/CAS.13009

[7] Huang J-S, Yang C-M, Wang J-S, Liou H-H, Hsieh I-C, Li G-C, et al. Caspase-3 expression in tumorigenesis and prognosis of buccal mucosa squamous cell carcinoma. Oncotarget. 2017; 8(48): 84237–84247. https://doi.org/10.18632/ONCOTARGET.20494

[8] Joseph B, Ekedahl J, Lewensohn R, Marchetti P, Formstecher P, Zhivotovsky B. Defective caspase-3 relocalization in non-small cell lung carcinoma. Oncogene. 2001;20(23): 2877–2888. https://doi.org/10.1038/SJ.ONC.1204402

[9] Takata T, Tanaka F, Yamada T, Yanagihara K, Otake Y, Kawano Y, et al. Clinical significance of caspase-3 expression in pathologic-stage I, nonsmall-cell lung cancer. International Journal of Cancer. 2001a; 96 Suppl(SUPPL.); 54–60. https://doi.org/10.1002/IJC.10347

[10] Anagnostou VK, Lowery FJ, Zolota V, Tzelepi V, Gopinath A, Liceaga C, Panagopoulos N, et al. High expression of BCL-2 predicts favorable outcome in non-small cell lung cancer patients with non squamous histology. BMC Cancer. 2010;*10*(1): 1–11. https://doi.org/10.1186/1471-2407-10-186/TABLES/2

[11] Sun PL, Sasano H, Gao H. Bcl-2 family in non-small cell lung cancer: its prognostic and therapeutic implications. Pathology International. 2017; 67(3): 121–130. https://doi.org/10.1111/PIN.12507

[12] Cakir E, Yilmaz A, Demirag F, Oguztuzun S, Sahin S, Yazici UE, Aydin M. Prognostic significance of micropapillary pattern in lung adenocarcinoma and expression of apoptosis-related markers: caspase-3, bcl-2, and p53. APMIS : Acta Pathologica, Microbiologica, et Immunologica Scandinavica. 2011; 119(9): 574–580. https://doi.org/10.1111/J.1600-0463.2011.02778.X

[13] Kim C H, Lee H S, Park JH, Choi JH, Jang SH, Park YB, et al. Prognostic role of p53 and Ki-67 immunohistochemical expression in patients with surgically resected lung adenocarcinoma: a retrospective study. Journal of Thoracic Disease. 2015; 7(5): 822. https://doi.org/10.3978/J.ISSN.2072-1439.2015.05.02

[14] Zhu CQ, Shih W, Ling CH, Tsao MS. Immunohistochemical markers of prognosis in non-small cell lung cancer: a review and proposal for a multiphase approach to marker evaluation. Journal of Clinical Pathology. 2006a; *59*(8): 790–800. https://doi.org/10.1136/JCP.2005.031351

[15] Ma X, Le Teuff G, Lacas B, Tsao MS, Graziano S, Pignon JP, et al . Prognostic and Predictive Effect of TP53 Mutations in Patients with Non-Small Cell Lung Cancer from Adjuvant Cisplatin-Based Therapy Randomized Trials: A LACE-Bio Pooled Analysis. Journal of Thoracic Oncology : Official Publication of the International Association for the Study of Lung Cancer.2016;11(6): 850–861. https://doi.org/10.1016/J.JTHO.2016.02.002

[16] Zhu CQ, Shih W, Ling CH, & Tsao MS. Immunohistochemical markers of prognosis in non-small cell lung cancer: a review and proposal for a multiphase approach to marker evaluation. Journal of Clinical Pathology. 2006b; *59*(8): 790. https://doi.org/10.1136/JCP.2005.031351

[17] Singh RR, Reindl KM. Glutathione S-Transferases in Cancer. Antioxidants (Basel, Switzerland). 2021;10(5): 701. https://doi.org/10.3390/ANTIOX10050701

[18] Zeng B, Ge C, Li R, Zhang Z, Fu Q, Li Z, Lin Z, Liu L, Xue Y, Xu Y, He J, Guo H, Li C, Huang W, Song X, & Huang Y. Knockdown of microsomal glutathione S-transferase 1 inhibits lung adenocarcinoma cell proliferation and induces apoptosis. Biomedicine & Pharmacotherapy. 2020; *121*: 109562. https://doi.org/10.1016/J.BIOPHA.2019.109562

[19] Albarakati N, Khayyat D, Dallol A, Al-Maghrabi J, & Nedjadi T.The prognostic impact of GSTM1/GSTP1 genetic variants in bladder Cancer. BMC Cancer. 2019; *19*(1): 1–11. https://doi.org/10.1186/S12885-019-6244-6/TABLES/5

[20] Chen Y, Li B, Wang J, Liu J, Wang Z, Mao Y, Liu S, Liao X, Chen J. Identification and verification of

the prognostic value of the glutathione S-transferase Mu genes in gastric cancer. Oncology Letters. 2020; 20(4): https://doi.org/10.3892/OL.2020.11961

[21] Hsu CH, Chen CL, Hong RL, Chen KL, Lin JF, Cheng AL.Prognostic value of multidrug resistance 1, glutathione-S-transferase-pi and p53 in advanced nasopharyngeal carcinoma treated with systemic chemotherapy. Oncology. 2002;62(4):305–312. https://doi.org/10.1159/000065061

[22] Huang J, Tan PH, Thiyagarajan J, Bay BH. Prognostic Significance of Glutathione S-Transferase-Pi in Invasive Breast Cancer. Modern Pathology.2003; 16(6): 558–565. https://doi.org/10.1097/01.mp.0000071842.83169.5a

[23] Voso M T, D'Alo F, Putzulu R, Mele L, Scardocci A, Chiusolo P, et al. (2002). Negative prognostic value of glutathione S-transferase (GSTM1 and GSTT1) deletions in adult acute myeloid leukemia. Blood. 2002; 100(8): 2703–2707. https://doi.org/10.1182/BLOOD.V100.8.2703

[24] Wang Y, Spitz MR, Schabath MB, Ali-Osman F, Mata H, & Wu X. Association between glutathione S-transferase p1 polymorphisms and lung cancer risk in Caucasians: a case-control study. Lung Cancer. 2003; 40(1): 25–32. https://doi.org/10.1016/S0169-5002(02)00537-8

[25] Pan C, Zhu G, Yan Z, Zhou Y, Liu Z. Glutathione S-Transferase T1 and M1 Polymorphisms Are Associated with Lung Cancer Risk in a Gender-Specific Manner. Oncology Research and Treatment. 2014;*37*(4): 164–169. https://doi.org/10.1159/000361083

[26] Wang SQ, Chen JJ, Jiang Y, Lei ZN, Ruan YC, Pan Y, et al. Targeting GSTP1 as Therapeutic Strategy against Lung Adenocarcinoma Stemness and Resistance to Tyrosine Kinase Inhibitors. Advanced Science (Weinheim, Baden-Wurttemberg, Germany). 2023; 10(7): https://doi.org/10.1002/ADVS.202205262

[27] Inoue T, Ishida T, Sugio K, Maehara Y, Sugimachi K. Glutathione S Transferase Pi Is a Powerful Indicator in Chemotherapy of Human Lung Squamous-Cell Carcinoma. Respiration. 1995;62(4): 223–227. https://doi.org/10.1159/000196451

[28] Rybárová S, Hodorová I, Mihalik J, Mirossay L. MRP1 and GSTp1 expression in non-small cell lung cancer does not correlate with clinicopathological parameters: A Slovakian population study. Acta Histochemica. 2014;116(8): 1390–1398. https://doi.org/10.1016/J.ACTHIS.2014.09.002

[29] Jankova L, Robertson G, Chan C, Tan KL, Kohonen-Corish M, Fung CLS, et al . Glutathione S-transferase Pi expression predicts response to adjuvant chemotherapy for stage C colon cancer: A matched historical control study. BMC Cancer. 2012;12(1): 1–9. https://doi.org/10.1186/1471-2407-12-196/TABLES/3

[30] Wang K, Zhang FL, Jia W. Glutathione S-transferase ω 1 promotes the proliferation, migration and invasion, and inhibits the apoptosis of non-small cell lung cancer cells, via the JAK/STAT3 signaling pathway. Molecular Medicine Reports, 2021; *23*(1): 1–7. https://doi.org/10.3892/MMR.2020.11709

[31] Mora Vidal R, Regufe da Mota S, Hayden A, Markham H, Douglas J, Packham G, Crabb SJ. Epidermal Growth Factor Receptor Family Inhibition Identifies P38 Mitogen-activated Protein Kinase as a Potential Therapeutic Target in Bladder Cancer. Urology. 2018; *112*: 225.e1-225.e7. https://doi.org/10.1016/J.UROLOGY.2017.10.041

[32] Zarczynska I, Gorska-Arcisz M, Cortez A J, Kujawa KA, Wilk A M, Skladanowski A C, Stanczak A, Skupinska M, Wieczorek M, Lisowska K M, Sadej R, & Kitowska K. P38 mediates resistance to fgfr inhibition in non-small cell lung cancer. Cells. 2021; 10(12): 3363. https://doi.org/10.3390/CELLS10123363/S1

[33] Fang Y, Wang J, Wang G, Zhou C, Wang P, Zhao S, et al. Inactivation of p38 MAPK contributes to stem cell-like properties of non-small cell lung cancer. Oncotarget. 2017; 8(16): 26702. https://doi.org/10.18632/ONCOTARGET.15804

[34] Sunaga N, Miura Y, Tsukagoshi Y, Kasahara N, Masuda T, et al. Dual inhibition of MEK and p38 impairs tumor growth in KRAS-mutated non-small cell lung cancer. Oncology Letters. 2019; 17(3): https://doi.org/10.3892/OL.2019.10009

[35] Feng C, Wu J, Yang F, Qiu M, Hu S, Guo S, Wu J, Ying X, Wang J. Expression of Bcl-2 is a favorable prognostic biomarker in lung squamous cell carcinoma. Oncology Letters. 2018; 15(5): 6925. https://doi.org/10.3892/OL.2018.8198

[36] Zhang J, Wang S, Wang L, Wang R, Chen S, Pan B, Sun Y, Chen H. Prognostic value of Bcl-2 expression in patients with non-small-cell lung cancer: a meta-analysis and systemic review. OncoTargets and Therapy. 2015; *8*: 3361. https://doi.org/10.2147/OTT.S89275

[37] Takata T, Tanaka F, Yamada T, Yanagihara K, Otake Y, Kawano Y, et al. Clinical significance of caspase-3 expression in pathologic-stage I, nonsmall-cell lung cancer. International Journal of Cancer. 2001b; 96 Suppl(SUPPL.), 54–60. https://doi.org/10.1002/IJC.10347

[38] Yoo J, Kim CH, Song SH, Shim BY, Jeong YJ, Ahn MI, et al. Expression of Caspase-3 and c-myc in Non-Small Cell Lung Cancer. Cancer Research and Treatment : Official Journal of Korean Cancer Association. 2004;36(5): 303. https://doi.org/10.4143/CRT.2004.36.5.303

[39] Sahu V, Mohan A, Dey S. p38 MAP kinases: plausible diagnostic and prognostic serum protein marker of non small cell lung cancer. Experimental and Molecular Pathology. 2019;107:118–123. https://doi.org/10.1016/J.YEXMP.2019.01.009

[40] Liu H, Tong X, Zhao H, Li Y, Zhang L. P2-080: Prognostic value of Smad4 and p38 proteins in Non-Small Cell Lung Cancer. Journal of Thoracic Oncology. 2007;2(8): S522. https://doi.org/10.1097/01.jto.0000283544.26342.98

[41] Ahrendt SA, Hu Y, Buta M, McDermot MP, Benoit N, Yang SC, et al. p53 mutations and survival in stage I non-small-cell lung cancer: results of a

prospective study. Journal of the National Cancer Institute. 2003;*95*(13):961–970. https://doi.org/10.1093/JNCI/95.13.961

[42] Mogi A, Kuwano H. TP53 mutations in nonsmall cell lung cancer. Journal of Biomedicine and Biotechnology. 2011;Jan18,2011:583929 https://doi.org/10.1155/2011/583929

[43] Bhattacharya P, Madden JA, Sen N, Hoyer PB, Keating AF. Glutathione S-transferase class μ regulation of apoptosis signal-regulating kinase 1 protein during VCD-induced ovotoxicity in neonatal rat ovaries. Toxicology and Applied Pharmacology. 2013;*267*(1): 49–56. https://doi.org/10.1016/J.TAAP.2012.12.013

[44] Dorion S, Lambert H, Landry J. Activation of the p38 signaling pathway by heat shock involves the dissociation of glutathione S-transferase Mu from Ask1. The Journal of Biological Chemistry.2002;277(34): 30792–30797. https://doi.org/10.1074/JBC.M203642200

[45] Luo HY, Tang WL, Xiang L, Peng LL, Wu D, Zhu ZY, Gu, HT, et al . Comprehensive analysis of the correlation between GSTM1 and tumor immunity in colon cancer. Journal of Gastrointestinal Oncology, 2022;13(6): 3025–3037. https://doi.org/10.21037/JGO-22-1060/COIF

[46] Jakubowska K, Guzińska-Ustymowicz K, Famulski W, Cepowicz D, Jagodzińska D, Pryczynicz A. Reduced expression of caspase-8 and cleaved caspase-3 in pancreatic ductal adenocarcinoma cells. Oncology Letters. 2016;11(3): 1879. https://doi.org/10.3892/OL.2016.4125

[47] Oguztüzun S, Aydin M, Demirag F, Yazici Ü, Özhavzali M, Kiliç M, Işcan M. The expression of GST isoenzymes and p53 in non-small cell lung cancer. Folia Histochemica et Cytobiologica. 2010;*48*(1): 122–127. https://doi.org/10.2478/V10042-008-0084-6

[48] Cancer Today. (n.d.). Retrieved February 19, from https://gco.iarc.fr/today/en, 2024.

[49] Scott A, Salgia R. Biomarkers in lung cancer: from early detection to novel therapeutics and decision making. Biomarkers in Medicine.2008;2(6): 577. https://doi.org/10.2217/17520363.2.6.577