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ORIGINAL ARTICLE

Clinicopathological Impact of CPA4, UHRF1, Glypican-1, and CD90 Expression in Lung Adenocarcinoma and Epithelioid Malignant Mesothelioma

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Abstract:

background: Accurate diagnosis, early detection, and differentiation of lung adenocarcinoma from mesothelioma; particularly the epithelioid subtype is needed to allow better management thus to improve their prognosis.Aim of our study was to assess applicability of CPA4, UHRF1, glypican-1 and CD90 expression by immunohistochemistry to differentiate between lung adenocarcinoma and epithelioid mesothelioma in addition to detection of their prognostic roles and relation to patients' survival.

Methods: We collected samples from 30 patients diagnosed with lung adenocarcinoma and 30 patients diagnosed with epithelioid mesothelioma. For the immunohistochemistry, sections of all collected samples were incubated with CPA4, UHRF1, glypican-1 and CD90 to assess their diagnostic accuracy.

RESULTS: A highly significant association was detected between positive CPA4, UHRF1 expression in lung adenocarcinoma and their diagnostic accuracy (p value <0.001). Also, highly significant association was detected between positive CD90, Glypican 1 expression in epithelioid mesothelioma and their diagnostic accuracy (p value <0.001). CPA4 expression was associated with shorter OS rate (P value 0.019, 0.009) UHRF1 expression was associated with shorter OS rate (P value=0.009)

Conclusions: CPA4, UHRF1, glypican-1 and CD90 were considered novel biomarkers that have important roles in differentiation between epithelioid malignant mesothelioma and adenocarcinoma of the lung with high sensitivity and specificity.

Keywords:
lung Adenocarcinoma, Epithelioid Mesothelioma, CPA4, UHRF1, Glypican-1, CD90

INTRODUCTION

Lung cancer is a potentially fatal disease which ranked as the 2nd commonest cancer in both males and females after breast and prostatic cancer respectively, forming the primary cause of cancer related fatality [1, 2].

Non small–cell lung cancer (NSCLC) forms the commonest histopathological subtype representing about 85% of all lung cancer subtypes and includes two main types which are squamous cell carcinoma and adenocarcinoma [3].

Many patients who were diagnosed with adenocarcinoma of the lung die within a short period after diagnosis and a small number of patients survive for years. Factors affecting prognosis and unfavorable outcome remain unknown [4].

Lung adenocarcinoma, particularly poorly differentiated cases, lacks early and accurate diagnosis which leads to late detection in advanced stages which lead to dismal clinical outcome [5].

Malignant mesothelioma is a highly aggressive malignant pleural tumor with a short median overall survival rate, and it forms a marked diagnostic challenge for pathologists for its diagnosis and differentiating it from lung adenocarcinoma [6].

Mesothelioma has 3 main histological subtypes; epithelioid (the commonest subtype), sarcomatoid and biphasic mesothelioma [7].

Accurate diagnosis, early detection and differentiation of lung adenocarcinoma from mesothelioma; particularly the epithelioid subtype is needed to allow better management thus to improve their prognosis [8].

There are many routinely used biomarkers for differentiation between lung adenocarcinoma and epithelioid mesothelioma Calretinin, WT1, D2-40, MOC31 and TTF1 but their specificity and sensitivity are not high enough to reach accurate diagnosis [9].

Novel, specific and sensitive markers are highly needed.

Carboxypeptidase A4 (CPA4) is carboxypeptidase A/B subfamily member which participated in carcinogenesis, progression, and aggressive behavior of many cancers [10,11].

CPA4 stimulated carcinogenesis, angiogenesis, invasion and metastases by protein kinase B(AKT)/c-MYC pathway activation and by controlling cancer stem cell behavior [12].

Ubiquitin-like with PHD and ring finger domains 1 (UHRF1) is a nuclear protein which is found in proliferating cells only, but it is not detected in quiescent cells [13].

UHRF1 upregulation was found in many cancers, additionally it was found to have a powerful diagnostic and prognostic roles in lung cancer [14].

Glypican-1 is a heparan sulfate proteoglycan family member that is commonly found at the cell surface and the extracellular matrix. Glypicans played many roles for cancer growth and progression [9].

CD90 was found to be a cancer stem cell marker in many cancers [15], but its role in diagnosis or progression of lung cancer is still not defined.

Aim of our study was to assess applicability of CPA4, UHRF1, glypican-1 and CD90 expression by immunohistochemistry to differentiate between lung adenocarcinoma and epithelioid mesothelioma in addition to detection of their prognostic roles and relation to patients' survival.

MATERIALS AND METHODS

Inclusion criteria

Cases with a sure diagnosis of lung adenocarcinoma and epithelioid mesothelioma, patients with sufficient samples in the paraffin blocks for immunohistochemistry and patients with complete clinical and follow-up data were included in our study.

Exclusion criteria

Cases diagnosed with other histopathological subtypes of lung carcinoma or mesotheliomas were excluded from the study.

Patients and tissue samples

We collected samples from 30 patients diagnosed with lung adenocarcinoma and 30 patients diagnosed with epithelioid mesothelioma. We collected all clinical data as (patients' age, sex, smoking status and co-morbid conditions), pathological data as (histopathological subtype, tumor grade, TNM stage and presence or absence of pleural effusion), and follow-up data as patients' survival, recurrence and response to therapy. All samples were collected from Zagazig university.

Immunohistochemistry

For the immunohistochemistry, sections of all collected samples were incubated with primary monoclonal anti-CPA4 antibody (1:100; Abcam, USA), primary mouse monoclonal anti-UHRF1 antibody (1: 1000, BD Bioscience), primary polyclonal anti-glypican-1 antibody (1:100 dilution; Proteintech Group, Rosemount, USA), and polyclonal anti- CD90 (Dako Cytomation). The results were assessed by three independent pathologists.

Evaluation of CPA4, UHRF1, glypican-1 and CD90 expression

We assessed cytoplasmic CPA4, glypican-1 and CD90 expression and nuclear UHRF1 expression semi-quantitatively in included tissues.

Staining intensity was classified as no staining (score = 0), weak staining (score = 1), moderate staining (score = 2) and strong staining (score = 3). Staining percentage was classified as no staining (0-5%), score 1 (5-50%), score 2 (50-75%) and score 3 (75-100%) [13, 15].

Final stain score of markers expression was obtained by multiplying the percentage and intensity scores of positively stained tumor cells giving scores from 0-9, we considered 3 as the cut point for positive stain of included markers above which is considered positive stain and below which is considered negative stain.

Statistical analysis:

Collected clinical, pathological, and oncological data were statistically analyzed using Statistical Package for Social Sciences (SPSS 24 Inc. Chicago, IL, USA) program. Testing of data for normal distribution was performed using the Shapiro Walk test. We represented qualitative data as frequencies and percentages. We used Chi square ($\chi 2$) and Fisher exact tests for calculating differences between qualitative variables. We expressed quantitative data as median and range.

Ethical approval:

Written informed consent was obtained from all participants. This study was approved by the Institutional Review Board (IRB) Zagazig University, Faculty of Medicine **(IRB number: ZU-IRB #10112/20-11/2022).** The study was done according to The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans **[16].**

Survival analysis

Kaplan Meier survival curves were used for estimation of overall and disease-free survival rates and comparing survival curves was done using log rank test.

Overall survival (OS): was defined as time from disease diagnosis to death or last follow up date. **Progression -free survival (PFS):** was defined as time from starting treatment to disease progression date.

P-value ≤ 0.05 indicates significant results, p <0.001 indicates highly significant results while, P> 0.05 indicates non-significant differences for all tests.

RESULTS:

Demographic, clinicopathological and prognostic parameters of included patients [Table1]:

We showed that 55% of our patients were older than 45 years old (p value **=0.003),** 60% of them were males (p value **=0.002),** 33.00% were smoker (p value= **0.005) ,** 73.30% showed positive lymph node metastasis, evidence of distant metastasis was detected in 36.70% of cases (p value= **0.032)**, 58.3% of patients showed partial response to therapy and 25% showed stable disease (p value **=0.016).**

CPA4, UHRF1, CD90 and glypican-1expression in samples of included patients [Table 2, figures 1 and 2.

We showed that samples from 50/60 (83.3%%) lung adenocarcinoma cases versus only two mesothelioma cases showed diffuse strong cytoplasmic CPA4 expression**, samples** from 54/60 (90%) lung adenocarcinoma cases versus only two mesothelioma cases showed nuclear UHRF1 expression**,** 58/60 (96.7%) mesothelioma cases versus four lung adenocarcinoma cases showed cytoplasmic Glypican 1 expression**,** 54/60 (90%) mesothelioma cases versus six lung adenocarcinoma showed cytoplasmic CD 90 expression**.** All with highly statistically significant association (p value**<0.001)**

Diagnostic accuracy of CPA4, UHRF1, CD90 and glypican-1expression in differentiation between lung adenocarcinoma and epithelioid mesothelioma [Table 3]

A highly significant association was detected between positive CPA4, UHRF1 expression in lung adenocarcinoma and their diagnostic accuracy (p value <0.001).

Also, highly significant association was detected between positive CD90, Glypican 1 expression in epithelioid mesothelioma and their diagnostic accuracy (p value <0.001).

Prognostic values of CPA4, UHRF1, CD90 and glypican-1expression in patients with lung adenocarcinoma and epithelioid mesothelioma and association with progression-Free survival and overall survival rates [Table 4, Figures 3].

Significant overall survival difference was found regarding some markers' expression; for CPA4 expression (P value of PFS in lung adenocarcinoma was 0.038 versus 0.006 in epithelioid mesothelioma)**,** for UHRF1 expression (P value of PFS in lung adenocarcinoma was 0.008 versus 0.586 in epithelioid mesothelioma)**,** for CD90 expression (P value of PFS in lung adenocarcinoma was 0.128 versus 0.325 in epithelioid mesothelioma).

For Glypican1 expression (P value of PFS in lung adenocarcinoma was 0.226 versus 0.586 in epithelioid mesothelioma)**.**

CPA4 expressions were independently associated with shorter OS in both adenocarcinoma and mesothelioma (P value 0.019, 0.009)

UHRF1 expression was independently associated with shorter OS in adenocarcinoma (P value=0.009) and not in mesothelioma

CD90 expression was not associated with OS in both adenocarcinoma and mesothelioma.

Glypican 1 expression was not associated with OS in both adenocarcinoma and mesothelioma.

Table 1: Demographic, clinicopathological and prognostic parameters of included patients

Characteristics		Histopathological subtype				Total N=120		p
		lung adenocarcinoma N=60		epithelioid mesothelioma N=60				
		N	%	N	%	N	%	
Age group	<45 years	8	13.30%	46	76.70%	54	45.00%	**0.003**
	≥45 years	52	86.70%	14	23.30%	66	55.00%	
Sex	Male	30	50.00%	42	70.00%	72	60.00%	**0.002**
	Female	30	50.00%	18	30.00%	48	40.00%	
Comorbidities	No	24	40.00%	38	63.30%	62	51.70%	0.071
	Yes	36	60.00%	22	36.70%	58	48.30%	
Smoking	No	30	50.00%	50	83.30%	80	67.00%	**0.005**
	Yes	30	50.00%	10	16.70%	40	33.00%	
Grade	I	10	16.70%	14	23.30%	24	20.00%	0.716
	II	40	66.70%	34	56.70%	74	61.70%	
	III	10	16.70%	12	20.00%	22	18.30%	
Size	5-7cm	4	6.70%	34	56.70%	38	31.70%	**0.021**
	>7cm	56	93.30%	26	43.30%	82	68.30%	
Site	Upper lobe	12	20.00%	22	36.70%	34	28.30%	0.202
	Middle lobe	32	53.30%	16	26.70%	48	40.00%	
	Lower Lobe	12	20.00%	16	26.70%	28	23.30%	
	All lung or Pleura	4	6.70%	6	10.00%	10	8.30%	
Malignant pleural or pericardial effusions	No	44	73.30%	8	13.30%	52	43.00%	0.007
	Yes	16	26.70%	52	86.70%	68	57.00%	
Stage	Stage IIB	20	33.30%	14	23.30%	34	28.30%	0.003
	Stage IIIA	2	3.30%	26	43.30%	28	23.30%	
	Stage IIIB	8	13.30%	6	10.00%	14	11.70%	
	Stage IV	30	50.00%	14	23.30%	44	36.70%	
LN metastasis	Negative	22	36.70%	10	16.70%	32	26.70%	0.08
	Positive	38	63.30%	30	83.30%	88	73.30%	
Distant metastases	No	30	50.00%	46	76.70%	76	63.30%	**0.032**
	Yes	30	50.00%	14	23.30%	44	36.70%	
M	M0	30	50.00%	46	76.70%	76	63.30%	0.098
	M1a	10	16.70%	4	6.70%	14	11.70%	
	M1b	20	33.30%	10	16.70%	30	25.00%	
Response to treatment	PD	14	23.30%	6	10.00%	20	16.70%	**0.016**
	SD	22	36.70%	8	13.30%	30	25.00%	
	PR	24	40.00%	46	76.70%	70	58.30%	
Response to treatment	NR	20	33.30%	10	16.70%	30	25.00%	0.136
	OAR	40	66.70%	50	83.30%	90	75.00%	
Progression	No	32	53.30%	46	76.70%	78	65.00%	0.058
	Yes	28	46.70%	14	23.30%	42	35.00%	
Death	Alive	28	46.70%	34	56.70%	62	51.70%	0.438
	Dead	32	53.30%	26	43.30%	54	48.30%	

PR: partial clinical response; SD: stable disease; PD: persistent disease; NR: no response ,OAR

Table 2: CPA4, UHRF1, CD90 and glypican-1expression in samples of included patients

Marker expression		Histopathological subtype				Total N=120		p
		lung adenocarcinoma N=60		epithelioid mesothelioma N=60				
		N	%	N	%	N	%	
CPA4	Negative	10	16.7%	58	96.7%	68	56.7%	**<0.001**
	Positive	50	83.3%	2	3.3%	52	43.3%	
UHRF1	Negative	6	10.0%	58	96.7%	64	53.3%	**<0.001**
	Positive	54	90.0%	2	3.3%	56	46.7%	
CD90	Negative	54	90.0%	6	10.0%	60	50.0%	**<0.001**
	Positive	6	10.0%	54	90.0%	60	50.0%	
glypican-1	Negative	56	93.3%	2	3.3%	58	48.3%	**<0.001**
	Positive	4	6.7%	58	96.7%	62	51.7%	

Table 3: Diagnostic accuracy of CPA4, UHRF1, CD90 and glypican-1expression in differentiation between *lung adenocarcinoma and* epithelioid mesothelioma

Marker expression		Histopathological subtype				Total N=120		p	Sensitivity (95% CI)	Specificity (95% CI)	AUC (95% CI)	PPV (95% CI)	NPV (95% CI)
		lung adenocarcinoma N=60		epithelioid mesothelioma N=60									
		N	%	N	%	N	%						
CPA4*	Negative	10	16.70%	58	96.70%	68	56.70%	**<0.001**	93.33% (85.279% - 94.358%)	96.67% (82.783% - 99.916%)	0.9 (0.795 - 0.962)	96.15% (78.333% - 99.425%)	85.29% (72.211% - 92.829%)
	Positive	50	83.30%	2	3.30%	52	43.30%						
UHRF1*	Negative	6	10.00%	58	96.70%	64	53.30%	**<0.001**	90.00% (73.471% - 97.888%)	96.67% (82.783% - 99.916%)	0.933 (0.838 - 0.982)	96.43% (79.659% - 99.466%)	90.63% (76.729% - 96.592%)
	Positive	54	90.00%	2	3.30%	56	46.70%						
CD90£	Negative	54	90.00%	6	10.00%	60	50.00%	**<0.001**	95.00% (82.107% - 96.793%)	90.00% (73.471% - 97.888%)	0.875 (0.751 - 0.952)	85.00% (65.597% - 94.395%)	90.00% (75.899% - 96.258%)
	Positive	6	10.00%	54	90.00%	60	50.00%						
glypican-1 £	Negative	56	93.30%	2	3.30%	58	48.30%	**<0.001**	96.67% (82.783% - 99.916%)	93.33% (77.926% - 99.182%)	0.95 (0.861 - 0.990)	93.55% (79.143% - 98.227%)	96.55% (80.262% - 99.484%)
	Positive	4	6.70%	58	96.70%	62	51.70%						

PPV (Positive Predictive Value), NPV (Negative Predictive Value)

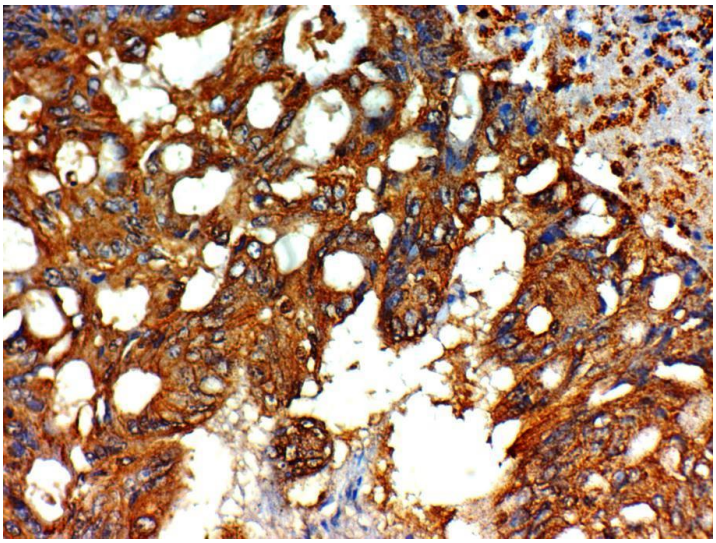
* Diagnostic accuracy to ***detect lung adenocarcinoma***
£ Diagnostic accuracy to ***detect epithelioid mesothelioma.***

Table 4: Prognostic values of CPA4, UHRF1, CD90 and glypican-1expression in patients with *lung adenocarcinoma and* epithelioid mesothelioma and association with progression-Free survival and overall survival rates

Marker	Histopathological subtype		Progression-Free Survival Analysis					Overall Survival Analysis				
			N of Events	Censored		PFS Rate %	P	N of Events	Censored		OS Rate%	P
				N	Percent				N	Percent		
CPA4	lung adenocarcinoma (N=60)	Negative (N=10)	0	10	100.0%	100 %	**0.038**	0	5	100.0%	100%	**0.019**
		Positive (N=30)	28	22	44.0%	42.0 %		16	9	36.0%	31.5%	
	epithelioid mesothelioma (N=60)	Negative (N=58)	14	44	75.9%	74.5 %	**0.006**	13	16	55.2%	54.6%	**0.009**
		Positive (N=2)	0	2	100.0%	100 %		0	1	100.0%	100%	
UHRF1	adenocarcinoma (N=60)	Negative (N=6)	0	6	100.0%	100 %	**0.008**	0	3	100.0%	100%	**0.009**
		Positive (N=54)	28	26	48.1%	46.6 %		16	11	40.7%	37.4%	
	mesothelioma (N=60)	Negative (N=58)	14	44	75.9%	74.5 %	**0.586**	13	16	55.2%	54.6%	**0.439**
		Positive (N=2)	0	2	100.0%	100 %		0	1	100.0%	100%	
CD90	adenocarcinoma (N=60)	Negative (N=54)	28	26	48.1%	46.6 %	**0.128**	16	11	40.7%	37.4%	**0.09**
		Positive (N=6)	0	6	100.0%	100 %		0	3	100.0%	100%	
	mesothelioma (N=60)	Negative (N=6)	0	6	100.0%	100 %	**0.325**	0	3	100.0%	100%	**0.16**
		Positive (N=54)	14	40	74.1%	72.4 %		13	14	51.9%	51.2%	
glypican-1	adenocarcinoma (N=60)	Negative (N=56)	28	28	50.0%	48.6 %	**0.226**	16	12	42.9%	40.0%	**0.18**
		Positive (N=4)	0	4	100.0%	100 %		0	2	100.0%	100%	
	mesothelioma (N=60)	Negative (N=2)	0	2	100.0%	100 %	**0.586**	0	1	100.0%	100%	**0.439**
		Positive (N=58)	14	44	75.9%	74.5 %		13	16	55.2%	54.6%	

FIGURES

A



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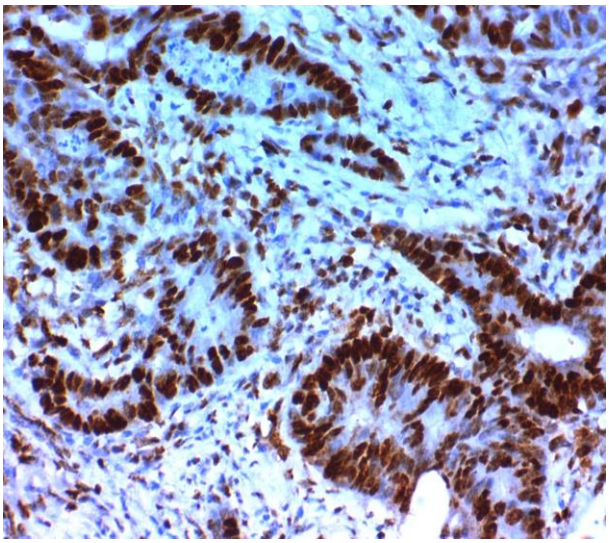

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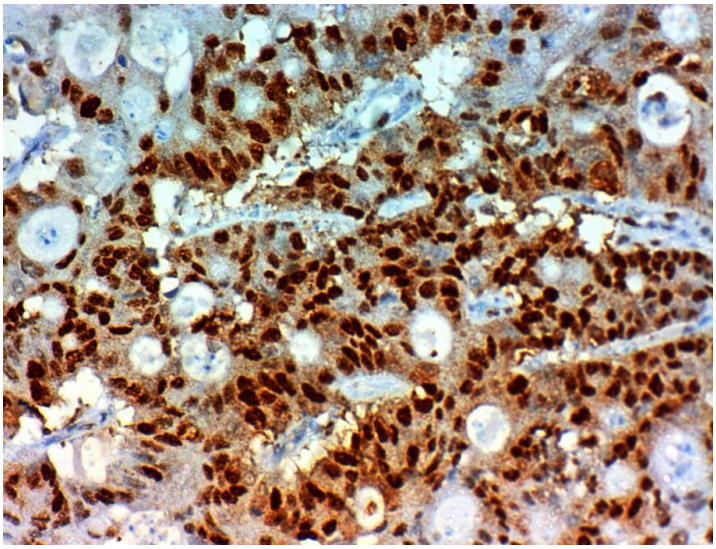

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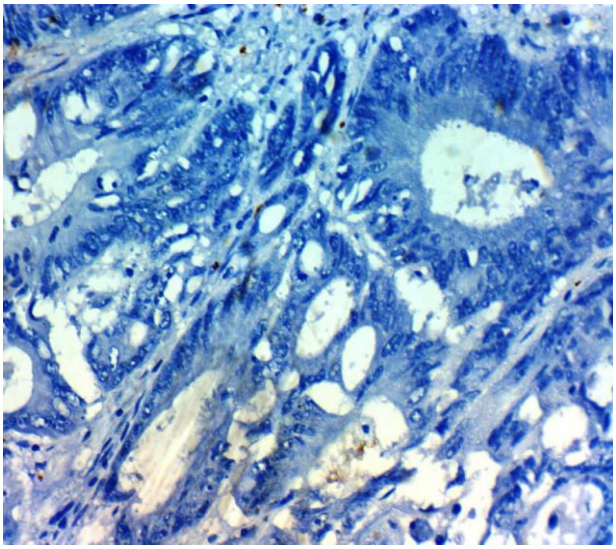

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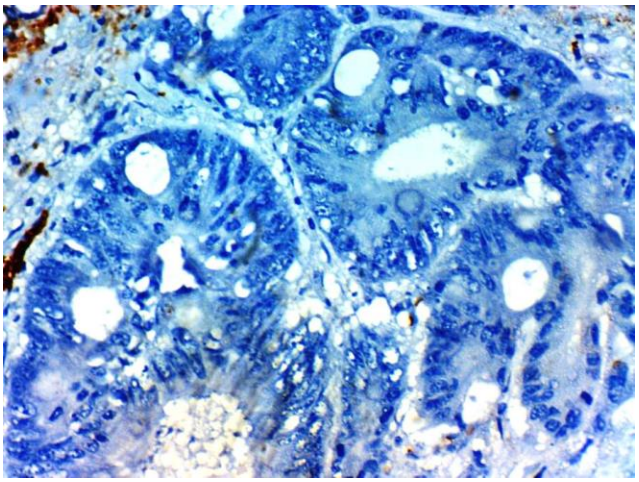


Figure 1: (A) lung adenocarcinoma grade II with tubular formation shows diffuse strong cytoplasmic CPA4 expression (+3) (original magnification x 400)
(B) lung adenocarcinoma grade III shows strong nuclear UHRF1 expression (+3) (IHC stain, original magnification x 400)
(C) Nests of lung adenocarcinoma cells grade III shows strong nuclear UHRF1 expression (+3) (original magnification x 400)
(D) lung adenocarcinoma grade II with tubular pattern negative for Glypican 1 (original magnification x 400)
(E) lung adenocarcinoma grade III negative for CD90 (original magnification x 400).

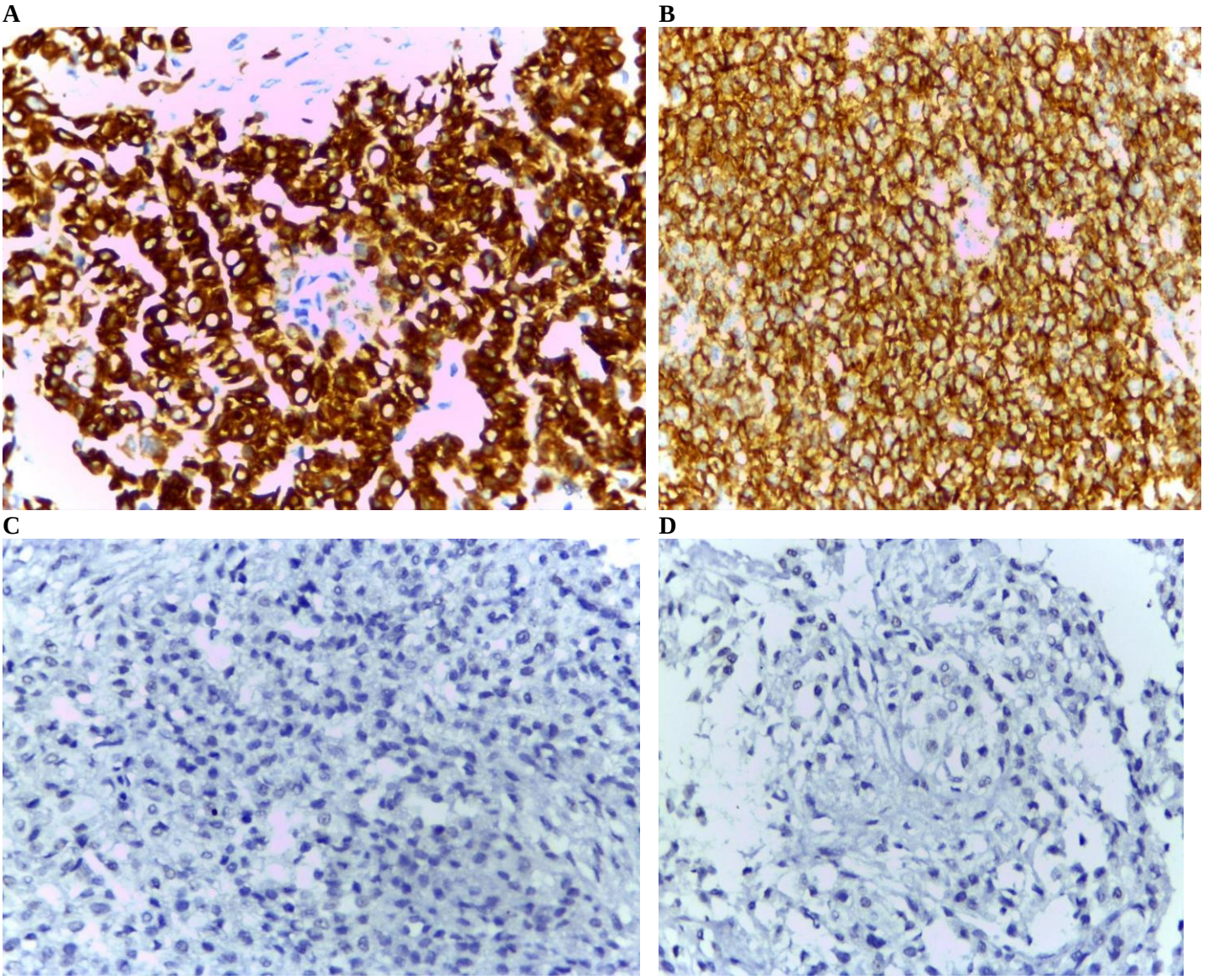
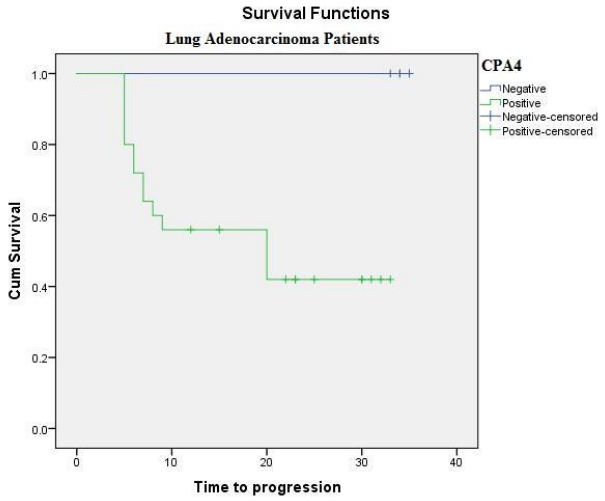


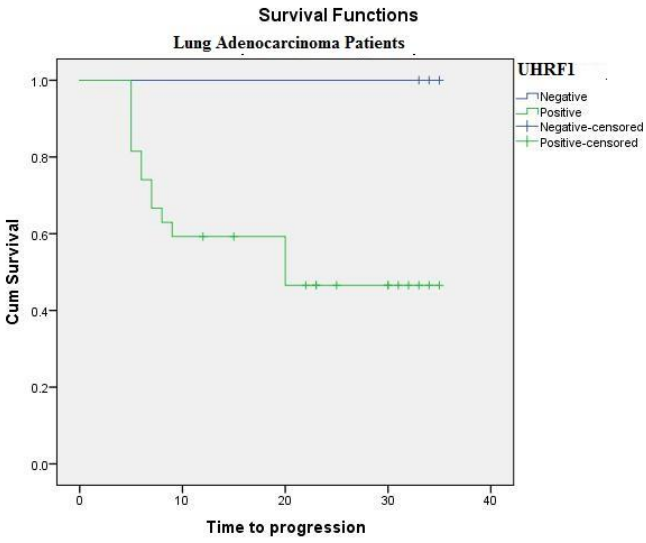
Figure 2
(A) Epithelioid mesothelioma grade III with ribbon like pattern shows diffuse strong cytoplasmic Glypican 1 expression (+3) (original magnification x 400)
(B) Diffuse sheets of Epithelioid mesothelioma grade III show strong cytoplasmic and membranous CD90 expression (+3) (original magnification x 400)
(C) Epithelioid mesothelioma grade III shows diffuse sheets of malignant cells with pleomorphic hyperchromatic nuclei negative for CPA4 (original magnification x 400)
(D) Epithelioid mesothelioma grade III negative for UHRF1 (original magnification x 400)

Fig. 3

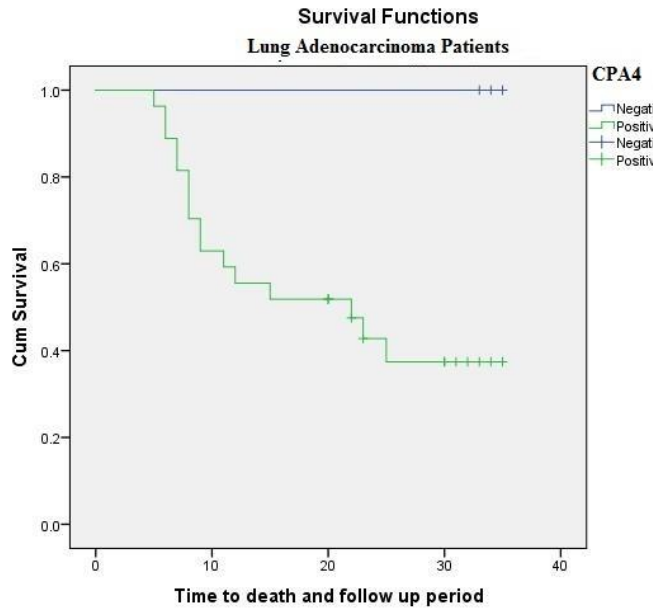
A


Survival Functions
Lung Adenocarcinoma Patients
CPA4
Negative
Positive
Negative-censored
Positive-censored
Cum Survival
Time to progression

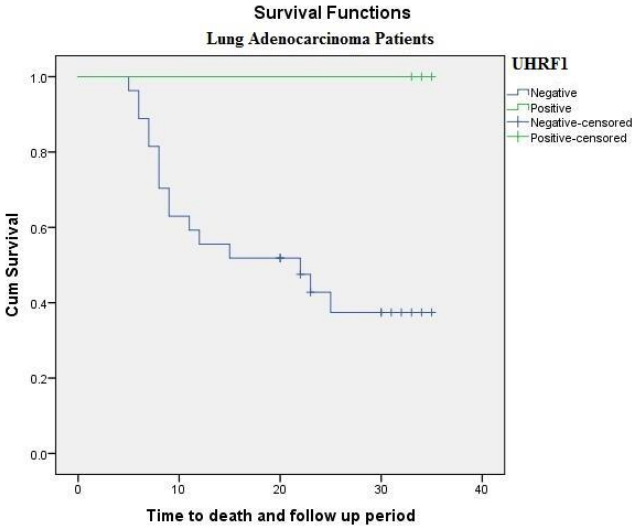
B


Survival Functions
Lung Adenocarcinoma Patients
UHRF1
Negative
Positive
Negative-censored
Positive-censored
Cum Survival
Time to progression

C


Survival Functions
Lung Adenocarcinoma Patients
CPA4
Cum Survival
Time to death and follow up period

D


Survival Functions
Lung Adenocarcinoma Patients
UHRF1
Negative
Positive
Negative-censored
Positive-censored
Cum Survival
Time to death and follow up period

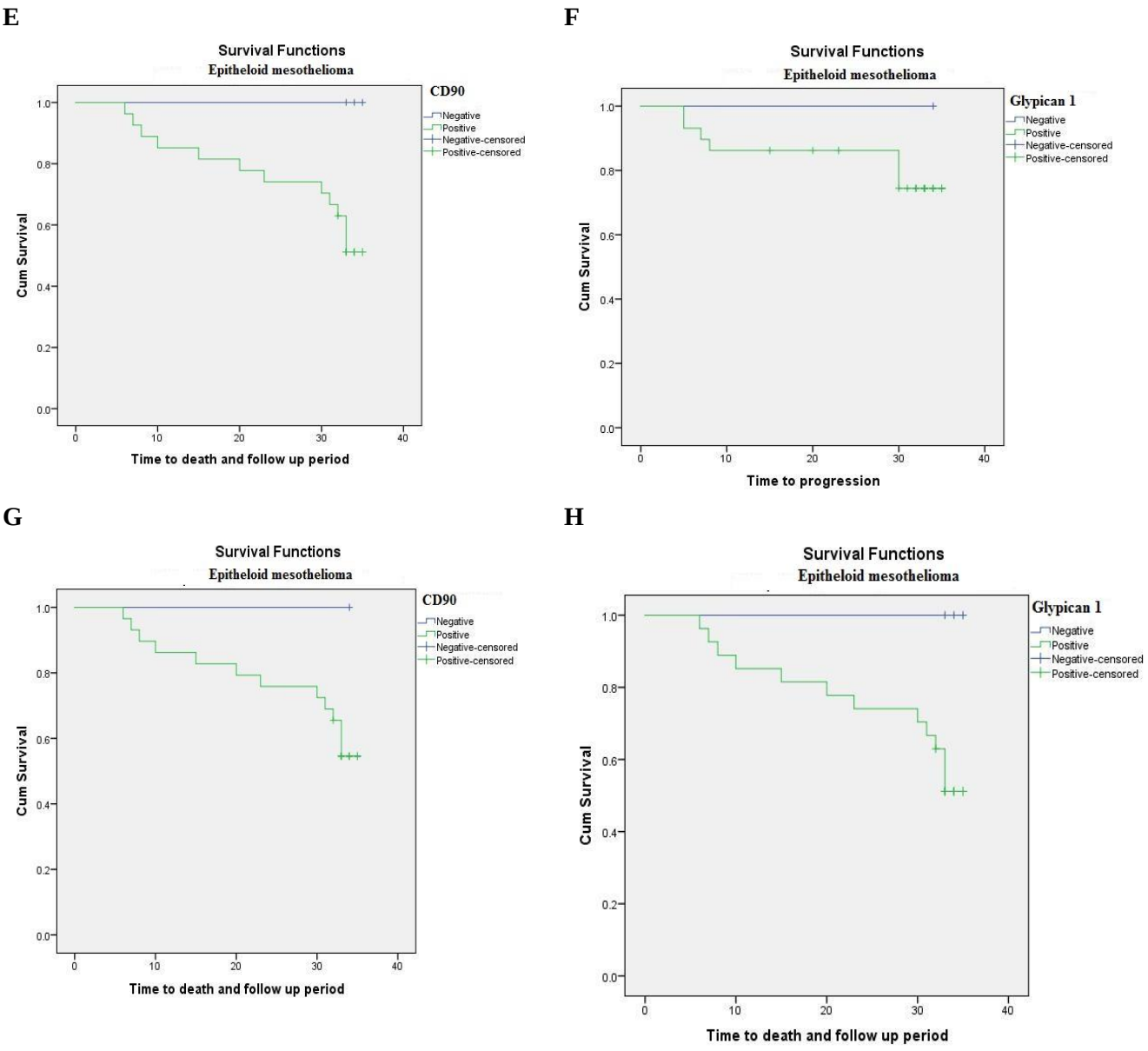


Figure 3:
- (A and B) progression free survival (PFS) rate of patients in association with SPA4 and UHRF1 expression (survival rates of patients with lung adenocarcinoma)
- (C and D) overall survival (OS) rate in association with SPA4 and UHRF1 expression. (survival rates of patients with lung adenocarcinoma)
- (E and F) progression free survival (PFS) rate of patients in association with CD90 and Glypican 1 expression. (survival rates of patients with epithelioid mesothelioma)
-(G and H) overall survival (OS) rate in association with CD90 and Glypican 1 expression. (survival rates of patients with epithelioid mesothelioma)

DISCUSSION:

Differentiation between malignant mesothelioma, particularly epithelioid subtype and adenocarcinoma of the lung is essential for accurate management of both cancers due to different prognoses and management protocols. Depending only on histopathology is not sufficient for accurate differential diagnosis [6].

CPA4 overexpression was found in several cancers, and is associated with the diagnosis and prognosis, but its role in lung cancer is still not clear [17].

In the current study we showed that CPA4 expression was found in most tissue samples of lung adenocarcinoma patients (83.3%) with high sensitivity and specificity (93.33% and 96.67% respectively), while its expression was detected only in one case of epithelioid mesothelioma which clarified its diagnostic role for lung adenocarcinoma and differentiating it from epithelioid mesothelioma.

Our findings are slightly similar to results of Sun et al (17) who reported that expression of CPA4 is elevated in lung adenocarcinoma tissues suggesting its valuable diagnostic role for lung adenocarcinoma.

Previous studies reported expression of CPA4 expression in 72.7% and 57.5 % of lung adenocarcinoma cases which is similar to our results in proving the diagnostic role of such biomarker in lung adenocarcinoma. The variety in number of positive cases between our results and their results was due to differences in number of included cases or type of used antibodies in our studies and their studies [18, 19].

Regarding the prognostic role of CPA4 we showed that its high expression in lung adenocarcinoma was associated with unfavorable OS and PFS survival rates. These results were in line with results of **Wang et al., [10]** that showed that high CPA4 levels were associated with unfavourable OS rate in lung cancer. Therefore, in addition to the diagnostic role of CPA4 its overexpression could be considered an unfavorable prognostic marker in patients with adenocarcinoma of the lung.

Although **Handa et al [20]** showed similar results to ours regarding the association between CPA4 expression and OS rate of breast cancer patients but they showed no association between CPA4 expression and DFS rate of patients.

We assessed the expression of another novel biomarker; UHRF1, which is a master of epigenetic silencing in several cancer tissues [21]. We showed that expression of UHRF1 was found in 90% of lung adenocarcinoma cases with high sensitivity and specificity, while its expression was positive in only one case of epithelioid mesothelioma.

Our results were similar to results of previous studies which showed the diagnostic role of UHRF1 due to its higher expression in lung cancer tissues more than normal lung tissues [22, 23].

Moreover, Unoki et al. [24] showed similar results to ours that UHRF1 was highly expressed in early stages lung adenocarcinoma patients which showed that UHRF1 could be considered a suitable diagnostic marker of lung cancer even in cases detected in the early stage. Additionally, combination of UHRF1 with other established diagnostic biomarkers could yield better results.

In addition to the diagnostic role of UHRF1 in lung adenocarcinoma and differentiating it from epithelioid mesothelioma, we showed that its high expression was associated with high grade, advanced stage and presence of lymph nodes metastases which is similar to results of previous studies [22, 23,24]

Different results were reported by **Daskalos et al [25]** who found no association between UHRF1 expression and prognostic parameters or patients' outcome.

Previous studies support our findings about the association between UHRF1 expression and inverse patients' outcome by showing that down regulation of UHRF1 induces arrest of the cell cycle and stimulating apoptosis in cancer cells [23]. Other studies demonstrated a controversy about its prognostic significance in cancer as it was found that UHRF1 downregulation might lead to an increase in degree of malignancy by activation of epithelial-mesenchymal transition (EMT) [26].

These results collectively showed that the prognostic role of UHRF1 expression and its effects on cell cycle and EMT depend on type of the tumor and is considered cell-type specific [27].

In the current study we showed an association between high UHRF1 expression, unfavorable survival and poor response to therapy, similarly previous studies showed a positive association between UHRF1 expression, PFS, OS rates, high incidence of tumor recurrence and unfavorable outcome [23, 28-30].

Our work reported no association between CPA4 expression, UHRF1 expression and outcome in mesothelioma patients as only a few cases showed positivity.

Regarding the expression of Glypican-1, we noted that it was expressed in 96.7 % of cases of epithelioid mesotheliomas, while it was negative in most cases of lung adenocarcinoma suggesting its significant role in mesothelioma diagnosis and differentiation between it and lung adenocarcinoma.

Our results were in line with **Amatya et al**. [7] who found that glypican-1 was considered a sensitive

and specific biomarker for epithelioid mesotheliomas and it could help in differentiating them from pulmonary adenocarcinoma.

Chiu et al. [32] showed different results that Glypican-1 has no role in differentiating mesothelioma from pulmonary adenocarcinoma as all included cases of mesothelioma and pulmonary adenocarcinoma in their study were positive for Glypican-1 expression. The cause of this discrepancy is that we used whole tissue sections for immunohistochemistry while Chiu et al. [32] used tissue microarrays.

We showed no significant association between Glypican-1 with patients' survival or response to therapy in either mesothelioma or lung adenocarcinoma cases.

Mesothelioma cancer stem cells are aggressive proliferating cells which are responsible for maintaining cancer cell proliferation and poor response to therapy [33].

In the current study we tried to evaluate the diagnostic and prognostic role of CD90 which is a cancer stem cell marker.

We showed that 90% of cases of mesothelioma were positive for CD90 while only 10% of adenocarcinoma cases were positive, suggesting its role in diagnosis of mesothelioma and differentiating it from adenocarcinoma. Similar results were showed by Kawamura et al. [34].

Slightly different results were found by **Sahin et al. [15]** that CD 90 overexpression was detected in 80% of mesothelioma cases and 63.3 % of pulmonary adenocarcinomas cases. These differences might be due to varieties of biopsy taking, different sample size and scoring system.

Previous studies showed that CD90 expression has many roles in cancer prognosis which depend on the type of cancer. It regulates cell proliferation, invasion, metastasis, and angiogenesis [35]. CD90 expression was associated with poor prognosis of ovarian cancer, breast cancer and lung cancer [35, 36].

We showed no association between CD90 expression, patients' survival or response to therapy in either mesothelioma nor adenocarcinoma cases.

Conclusions

In the present work we assessed the diagnostic roles of combinations of CPA4, UHRF1, CD90 and glypican-1 expression in differentiation between lung adenocarcinoma and epithelioid malignant mesothelioma mesothelioma and we showed that using these markers together raised the accuracy, sensitivity, and specificity of diagnosis to 100% even in small samples and early stages. Reaching accurate diagnosis is highly needed as both subtypes of malignant tumor have different lines of treatment and management strategies.

Points of strengths of the study

The studied markers and their association with diagnosis and patients' prognosis have not previously clarified together. Using whole tissue paraffin blocks not tissue microarray allows better detection of marker expression and diagnostic utility.

Recommendations:

Large prospective cohort studies are recommended to prove the diagnostic and prognostic roles of our novel markers in patients with lung adenocarcinoma and epithelioid mesothelioma. Additionally, we recommended evaluation of our markers in all histopathological sub-types of malignant mesothelioma and other subtypes of lung cancers.

Declarations:

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Conflicts of interest: the authors have no conflict of interest.

Ethics approval: The study was approved by the Institutional Review Board of Zagazig Faculty of Medicine and carried out according to the Declaration of Helsinki. **(ZU-IRB #10112/20-11/2022)**

Consent to participate: all participants provided written informed consent.

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Authors' contributions: All authors contributed to the study design. Dr. Doaa I. Abdelrahman, Dr. Mariem A. Elfeky and Dr Marwa M. El Mosely contributed to the study conception. Dr. Doaa Mandour, Dr. Rehab Hemeda and Dr. Mohamed M. Abozaid collected the samples and analyzed the data and contributed to writing the article. All authors participated in data analysis and contributed to drafting the paper, its critical revision and approved the final version to be submitted.

REFERENCES:

[1] Bade BC, Dela Cruz CS. Lung Cancer 2020: Epidemiology, Etiology, and Prevention. Clin Chest Med. 2020 Mar;41(1):1-24.

[2] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin. 2020 Jan;70(1):7-30.

[3] Relli V, Trerotola M, Guerra E, Alberti S. Abandoning the Notion of Non-Small Cell Lung Cancer. Trends Mol Med. 2019 Jul;25(7):585-594.

[4] Muppa P, Parrilha Terra SBS, Sharma A, Mansfield AS, Aubry MC, Bhinge K, et al. Immune Cell Infiltration May Be a Key Determinant of Long-Term Survival in Small Cell Lung Cancer. J Thorac Oncol. 2019 Jul;14(7):1286-1295.

[5] Xu K, Zhang C, Du T, Gabriel ANA, Wang X, Li X, et al. Progress of exosomes in the diagnosis and treatment of lung cancer. Biomed Pharmacother. 2021; 134:111111.

[6] Alì G, Bruno R, Fontanini G. The pathological and molecular diagnosis of malignant pleural mesothelioma: a literature review. J Thorac Dis. 2018 Jan;10(Suppl 2): S276-S284.

[7] Amatya VJ, Kushitani K, Kai Y, Suzuki R, Miyata Y, Okada M, et al. Glypican-1 immunohistochemistry is a novel marker to differentiate epithelioid mesothelioma from lung adenocarcinoma. Mod Pathol. 2018 May;31(5):809-815.

[8] Carbone M, Adusumilli PS, Alexander Jr HR, Baas P, Bardelli F, Bononi A, et al. Mesothelioma: Scientific clues for prevention, diagnosis, and therapy. CA: a cancer journal for clinicians. 2019 Sep;69(5):402-29.

[9] Li N, Gao W, Zhang YF, Ho M. Glypicans as cancer therapeutic targets. Trends Cancer. 2018 Nov;4(11): 741–54.

[10] Wang Y, Xie Y, Niu Y, Song P, Liu Y, Burnett J, et al. Carboxypeptidase A4 negatively correlates with p53 expression and regulates the stemness of breast cancer cells. Int J Med Sci. 2021 Feb 18;18(8):1753-1759.

[11] Shao Q, Zhang Z, Cao R, Zang H, Pei W, Sun T. CPA4 Promotes EMT in Pancreatic Cancer via Stimulating PI3K-AKT-mTOR Signaling. Onco Targets Ther. 2020 Aug 25; 13:8567-8580.

[12] Fu Y, Su L, Cai M, Yao B, Xiao S, He Q, et al. Downregulation of CPA4 inhibits non-small-cell lung cancer growth by suppressing the AKT/c-MYC pathway. Mol Carcinog. 2019 Nov;58(11):2026-2039.

[13] Jung HJ, Byun HO, Jee BA, Min S, Jeoun UW, Lee YK, et al. The Ubiquitin-like with PHD and Ring Finger Domains 1 (UHRF1)/DNA Methyltransferase 1 (DNMT1) Axis Is a Primary Regulator of Cell Senescence. J Biol Chem. 2017 Mar 3;292(9):3729-3739.

[14] Kong X, Chen J, Xie W, Brown SM, Cai Y, Wu K, et al. Defining UHRF1 Domains that Support Maintenance of Human Colon Cancer DNA Methylation and Oncogenic Properties. Cancer Cell. 2019 Apr 15;35(4):633-648.e7.

[15] Sahin N, Akatli AN, Celik MR, Ulutas H, Samdanci ET, Colak C. The role of CD90 in the differential diagnosis of pleural malignant mesothelioma, pulmonary carcinoma, and comparison with calretının. Pathology & Oncology Research. 2017 Jul;23(3):487-91.

[16] World Medical Association (2001) World Medical Association Declaration of Helsinki. Ethical principles for medical research involving human subjects. Bull. World Health Organ 79: 373-374.

[17] Sun L, Wang Y, Yuan H, et al. CPA4 is a Novel Diagnostic and Prognostic Marker for Human Non-Small-Cell Lung Cancer. J Cancer. 2016;7(10):1197-1204.

[18] Zhang, F, Zhang, Y, Sun, L-x, Chen, M, Ran, Y-l, Sun, L-c. Carboxypeptidase A4 promotes migration and invasion of lung cancer cells and is closely associated with lymph node metastasis. Prec Radiat Oncol. 2019; 3: 44– 51.

[19] Sun L., Guo C., Burnett J., Pan J., Yang Z., Ran Y., et al. Association between expression of carboxypeptidase 4 and stem cell markers and their clinical significance in liver cancer development. J. Cancer. 2017; 8:111–116.

[20] Handa T, Katayama A, Yokobori T, Yamane A, Fujii T, Obayashi S, et al. Carboxypeptidase A4 accumulation is associated with an aggressive phenotype and poor prognosis in triple-negative breast cancer. Int J Oncol. 2019 Mar;54(3):833-844.

[21] Polepalli S, George SM, Vidya RV, Rodrigues GS, Ramachandra L, Chandrashekar R, et al. Role of UHRF1 in malignancy and its function as a therapeutic target for molecular docking towards the SRA domain. The international journal of biochemistry & cell biology. 2019 Sep 1; 114:105558.

[22] Goto D, Komeda K, Uwatoko N, Nakashima M, Koike M, Kawai K, et al. UHRF1, a Regulator of Methylation, as a Diagnostic and Prognostic Marker for Lung Cancer. Cancer Invest. 2020 Apr;38(4):240-249.

[23] Tu Z, Deng X, Hou S, Feng A, Zhang Q. UHRF1 predicts poor prognosis by triggering cell cycle in lung adenocarcinoma. J Cell Mol Med. 2020 Jul;24(14):8069-8077.

[24] Unoki M, Daigo Y, Koinuma J, Tsuchiya E, Hamamoto R, Nakamura Y. UHRF1 is a novel

diagnostic marker of lung cancer. Br J Cancer. 2010 Jul 13;103(2):217-22.

[25] Daskalos, A., Oleksiewicz, U., Filia, A., Nikolaidis, G., Xinarianos, G., Gosney, J.R., et al. UHRF1-mediated tumor suppressor gene inactivation in non-small cell lung cancer. Cancer, 2011 ;117: 1027-1037.

[26] Jung YD, Shim JW, Park SJ, Choi SH, Yang K, Heo K, et al. Downregulation of UHRF1 promotes EMT via inducing CXCR4 in human cancer cells. Int J Oncol2015; 46:1232–1242. 2015.

[27] Liu X, Ou H, Xiang L, Li X, Huang Y, Yang D. Elevated UHRF1 expression contributes to poor prognosis by promoting cell proliferation and metastasis in hepatocellular carcinoma. Oncotarget. 2017 Feb 7;8(6):10510-10522.

[28] Tu Z, Chen X, Tian T, Chen G, Huang M. Prognostic significance of epigenetic regulatory gene expression in patients with non-small-cell lung cancer. Aging (Albany NY). 2021 Feb 26;13(5):7397-7415.

[29] Patnaik D, Estève PO, Pradhan S. Targeting the SET and RING-associated (SRA) domain of ubiquitin-like, PHD and ring finger-containing 1 (UHRF1) for anti-cancer drug development. Oncotarget. 2018 May 25;9(40):26243-26258.

[30] Yang C, Wang Y, Zhang F, Sun G, Li C, Jing S, et al. Inhibiting UHRF1 expression enhances radiosensitivity in human esophageal squamous cell carcinoma. Mol Biol Rep. 2013 Sep; 40(9):5225-35.

[31] Walker C, Mojares E, Del Río Hernández A. Role of Extracellular Matrix in Development and Cancer Progression. Int J Mol Sci. 2018 Oct 4;19(10):3028.

[32] Chiu K, Lee L, Cheung S, Churg AM. Glypican-1 immunohistochemistry does not separate mesothelioma from pulmonary adenocarcinoma. Mod Pathol. 2018 Sep;31(9):1400-1403.

[33] Wang D, Gao Y, Zhang Y, Wang L, Chen G. Glypican-3 promotes cell proliferation and tumorigenesis through up-regulation of β-catenin expression in lung squamous cell carcinoma. Biosci Rep. 2019 Jun 25;39(6): BSR20181147.

[34] Kandasamy S, Adhikary G, Rorke EA, Friedberg JS, Mickle MB, Alexander HR, et al. The YAP1 Signaling Inhibitors, Verteporfin and CA3, Suppress the Mesothelioma Cancer Stem Cell Phenotype. Mol Cancer Res. 2020 Mar; 18(3):343-351.

[35] Kawamura K, Hiroshima K, Suzuki T, Chai K, Yamaguchi N, Shingyoji M, et al. CD90 is a diagnostic marker to differentiate between malignant pleural mesothelioma and lung carcinoma with immunohistochemistry. American Journal of Clinical Pathology. 2013 Oct 1;140(4):544-9.

[36] Kumar A, Bhanja A, Bhattacharyya J, Jaganathan BG. Multiple roles of CD90 in cancer. Tumour Biol. 2016 Sep; 37(9):11611-11622.

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