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Original Article

Levels of APE1 Repair Gene and CLEC4M in Lung Cancer Patients Receiving Cisplatin Chemotherapy

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Abstract

This study aimed to detect the levels of apurinic/apyrimidinic endonuclease 1 (APE1) gene expression and C-type lectin domain family 4 member M (CLEC4M) and their association with cisplatin chemotherapy in lung cancer patients. Overall, 105 individuals who attended the Al-Amal National Hospital for Cancer Management, Baghdad, Iraq, were enrolled in the study and divided into three equal groups. The groups included the patients newly diagnosed with lung cancer, cancer patients who received cisplatin, and the healthy control group. All study groups were subjected to the sampling of the venous blood for molecular analysis by real-time polymerase chain reaction (RT-PCR) to detect the APE1 gene and enzyme-linked immunosorbent assay (ELISA) for serological testing to measure the concentration of CLEC4M protein. Significantly, the values of both cancer groups were higher than those reported in the control group. The relative index revealed a significant difference in the mean fold change level of APE1 in the newly diagnosed group (3 fold) and cisplatin therapy patients group (2 fold), compared to the control group (P=0.005). No significant differences were detected between the two cancer groups in terms of fold change mean of expression, demographic characteristics, and cancer histological type. Regarding human CLEC4M protein level, cases receiving cisplatin (139.2±25.9) and newly diagnosed patients (331.0±38.1) had a highly significant difference with the control group (100.3±47.5, P<0.001). There was no significant difference between the concentration level of CLEC4M and all parameters in demographic characteristics and cancer histological type. This was the first study to demonstrate that higher expression levels of new APE1, CLEC4M, and glutathione, especially after chemotherapy, are beneficial as diagnostic and prognostic markers for resistance to platinum chemotherapy in Iraqi lung cancer patients.

Keywords: Apurinic/Apyrimidinic Endonuclease 1, Cisplatin, C-Type Lectin Domain Family 4 Member M, Platinum chemotherapy

1. Introduction

Cancer is considered one of the most increasing diseases and the second cause of death worldwide, particularly in low- and middle-income countries, as a result of behavioral and dietary risks, such as high body mass index, low fruit and vegetable intake, lack of physical activity, tobacco use, and alcohol use (1). Globally, many diagnostic assays have been used to detect the location and type of cancer; however, molecular techniques have revealed high advantages in the identification of specific genes, proteins, and other factors unique to a type of tumor (2).

Different therapeutic approaches have been applied for the treatment and control of the disease, such as radiotherapy, immunotherapy, and chemotherapy (3). Cisplatin is the first platinum-based chemotherapy,

which was approved by the Food and Drug Administration in 1978 to treat cancer patients by attacking all rapidly dividing cells, which led to severe side effects and induced the ability of cancers to develop drug resistance (4, 5). Cisplatin-induced DNA lesions can strongly induce cell death when these lesions are not adequately repaired or processed. Cisplatin-based chemotherapy is commonly used to treat patients with advanced non-small cell lung cancer (NSCLC); therefore, resistance leads to the failure of this type of chemotherapy (6). Response to chemotherapy medications is closely related to the regulation of the DNA repair system (7).

Apurinic/apyrimidinic endonuclease 1 (APE1) is an important multi-functional protein that acts as a base excision-repair enzyme and a redox co-activator of a number of important transcription factors (8, 9). Many studies have shown a significant increase in APE1 repair gene expression in various types of cancer, suggesting that this gene is associated with survival outcome, lymph node status, proliferation index, and resistance to chemotherapy or radiotherapy (10, 11). In addition, the C-type lectin domain family 4 member M (CLEC4M) protein is one of the potential molecular markers used to identify the early stage of cancer in serum and tissues since the high expression of CLEC4M is associated with a poorer prognosis (12). Results of one study revealed that patients with higher CLEC4M expression were attributable to enhanced cisplatin resistance (13). Another study reported that immune-related genes, including CLEC4M in lung squamous cell carcinoma, were influential in the recognition of disease progression and prognosis (14).

In Iraq, the online-available data for the investigation of the role of APE1 gene expression in lung cancer and protein expression in cervical cancer were scarce; however, there were no resources about the role of CLEC4M. Hence, this study aimed to evaluate the relationship between APE1 gene expression and levels of CLEC4M in lung cancer patients who received platinum-based chemotherapy. It also aimed to find the correlation of APE1 and CLEC4M with demographic parameters, including age, gender, smoking habits, and the histological type and stage of the disease.

2. Materials and Methods

2.1. Study Samples

In total, 105 individuals who had referred to the Al-Amal National Hospital for cancer management were selected for the present study and subjected to venous blood sampling. According to their health status, the study population was divided into three groups as follows:

1. The first group included 35 patients newly diagnosed with lung cancer between 20 and 45 years old.

2. The second group included 35 patients with lung cancer who received chemotherapy (cisplatin or one of its derivatives) between 26 and 75 years old.

3. The third group, which included 35 healthy individuals, was considered a control group. The ages of the study population in this group ranged from 26 to 75 years old.

Each sample contained 100 µl of whole blood in an Eppendorf tube containing 300 µl shield buffer reagent to estimate the APE1 repair gene molecularly. For comparison, 3 ml of whole blood was transferred into the free-anticoagulant glass gel tube and centrifuged at 3,000 rpm for 5 min. Afterward, the obtained sera were kept in labeled Eppendorf tubes that were frozen until used for serological estimation of the CLEC4M marker.

2.2. Molecular Assay

Real-time polymerase chain reaction (RT-PCR) was applied to estimate the gene expression of the APE1 repair gene. According to the instructions of the manufacturer, RNAs were extracted using the RNA extraction kit (Zymo, USA). Moreover, the cDNAs were synthesized using the PrimeScript™ RT reagent kit (Takara, Korea) with specific conditions involving reverse transcription at 37 ºC for 15 min, at 85 ºC for 5 s, and at 4 ºC to inactivate the reverse transcriptase with heat treatment. The sequences of the primer that targeted the APE1 gene were F: 5′-GAGTAAGACGGC CGCAAAGAAAAA-3′ and R:

5′- CCGAAGGAGCTGACCAGTATTGAT-3′. Moreover, the sequences of GAPDH as a reference gene were F: 5′- CACTAGGCGCTCACTGTTCTC-3′ and R: 5′- AATCCGTTG ACTCCGACCTT-3′ with product sizes of 128 bp and 89 bp, respectively.

The Mastermix tubes were prepared using the KAPA SYBR® FAST qPCR Master Mix (2×) kit (Takara, Korea) at a final volume of 20 µl, which were subjected to thermocycler using the SaCycler RT-PCR System (Sacace, Italy) with program conditions specific to the cDNA samples and reference gene, including denaturation at 95 ºC, annealing at 56-60 ºC, extension at 72 ºC for 20 sec and 40 cycles. The gene expression for APE1 repair and GAPDH genes were calculated using the comparative quantitative method $2^{-(\Delta\Delta Ct)}$.

2.3. Serological Assay

The sandwich enzyme-linked immunosorbent assay (ELISA) kit (MyBioSource, USA) was used to measure the concentration level of the CLEC4M marker in the sera of patients. Following the instructions of the manufacturer, the standards, samples, and reagents were prepared and subjected to the procedure of the kit. Afterward, 100 µl of Biotin conjugated detection antibody were added to the wells; subsequently, after washing with wash buffer, 100 µl of horseradish peroxidase (HRP)-streptavidin was added, and unbound conjugates were washed away. Afterward, 90 µl of tetramethylbenzidine (TMB) substrates were used to visualize HRP enzymatic reaction. The HRP catalyzed TMB to produce a blu-color product that changed into yellow after the addition of 50 µl of acidic stop solution. Optical density was read at 450 nm using the Automatic ELISA Reader (Paramedical Srl, Italy).

2.4. Statistical Analysis

All obtained data were analyzed statistically by the SPSS software (version 24.0, SPSS Inc., USA) using analysis of variance, t-test, and chi-squared test (x^2). The analyzed data were presented as mean±SD. Furthermore, the least significant difference was used for comparison among the three groups, and *P* values of less than 0.05 were considered statistically significant.

3. Results

3.1. Apurinic/Apyrimidinic Endonuclease 1 Gene Expression and Mean Fold Change

The expression of the APE1 gene was detected in all cancer cases that received cisplatin, cases that did not receive chemotherapy, and the healthy controls. The mean values of APE1 gene expression in cancer cases who received chemotherapy and the newly diagnosed cancer cases were 2.72±1.7 and 3.55±0.04, respectively. Significantly, the values of both cancer groups were higher than that reported in the control group population (1.5±1.7, $P \leq 0.005$). These findings indicated that the relative expression of APE1 in cancer cases who received cisplatin was two folds more than the normal population of the control group, while in newly diagnosed cases it was three folds higher than that in the control group (Table 1).

Table 1. *APE1* Relative index and fold change among the study groups

***APE1* relative index**	**Control**	**Newly diagnosed**	**Received Cisplatin**
Range	0.13 - 6.9	1.23 - 8.57	0.08 - 7.4
Mean± SD	1.50 ± 1.7	3.55 ± 0.04	2.72 ± 1.7
LSD		1.16	
P value		≤ 0.005	

Significant differences * (P<0.05)

3.2. Apurinic/Apyrimidinic Endonuclease 1 Gene Expression Associated with Demographic Characteristics and Histological Types of the Newly Diagnosed Group

Association between APE1 fold changes with the demographic characteristics in a group of newly diagnosed cancer patients showed a significant variation in its values (Table 2). However, increases were observed in patients aged < 50 (3.7±2.4), males (3.6±1.9), smokers (3.5±2.0), patients with adenocarcinoma (3.8±2.1) and LCC (4.9±2.5)

regarding histological types, and patients at stage 4a (5.3±2.5). No significant differences (P>0.05) were detected between the APE1 fold changes of the demographic characteristics, except for those of NSCLC types, which were significantly higher, compared to SCLC cancer histological types (P<0.05).

3.3. Apurinic/Apyrimidinic Endonuclease 1 Gene Expression Associated with Demographic Characteristics and Histological Types of the Cisplatin Chemotherapy Group

The findings of APE1 fold change related to the demographic characteristics of cancer patients who received cisplatin revealed increases in patients aged < 50 (2.3±1.51), males (2.2±1.8), smokers (2.4±1.8), patients with squamous cell carcinoma of the skin (2.3±1.76), and limited type of SCLC (6.3±1.3) regarding histological types, compared to patients with extensive cancers at stage 2b (2.5±3.36) and drug cycles 4-6 (2.2±1.9). No significant differences were detected between fold change mean of expression values of the demographic characteristics; except for the values of SCLC types that were significantly higher than those of NSCLC cancer histological types (P<0.05) in lung cancer patients (Table 3).

Table 2. Fold change mean of *APE1* mRNA expression level in different demographic criteria and cancer histological types in lung cancer newly diagnosed group

Parameter		Total No.	APE1 fold change	*P*-value
Age	<50	9	3.7 ± 2.4	0.739
	>50	26	3.4 ± 1.8	
Gender	Male	33	3.6 ± 1.9	0.135
	Female	2	1.5 ± 0.23	
Smoking	Smoker	31	3.5 ± 2.0	0.89
	Non-smoker	4	3.4 ± 1.6	
Histology NSCLC*	Adenocarcinoma	19	3.8 ± 2.1	0.151
	Squamous	7	2.5 ± 0.7	
	LCC	4	4.9 ± 2.5	
SCLC	Limited	5	2.8 ± 2.1	
	Extensive	0	-	
Stage	1	13	2.5 ± 1.1	0.113
	2 a	11	4.0 ± 2.1	
	3 b	6	4.1 ± 2.5	
	4 a	3	5.3± 2.5	
	5 a	2	2.6± 0.25	

Significant differences * (P<0.05)

Table 3. Fold change mean of *APE1* mRNA expression level in different demographic criteria and cancer histological in lung cancer received Cisplatin chemotherapy group

Parameter		Total No.	APE1 Fold change	P-value
Age	<50	8	2.3 ± 1.51	0.855
	>50	27	2.1 ± 1.38	
Gender	Male	32	2.2 ± 1.8	0.433
	Female	3	1.4 ± 0.16	
Smoking	Smoker	31	2.4 ± 1.8	0.816
	Non-smoker	4	2.1 ± 1.7	
Histology NSCLC	Adenocarcinoma	16	2.1 ± 1.42	0.561
	Squamous	6	2.3 ± 1.76	
	LCC	3	1.1 ± 0.52	
SCLC*	Limited	6	6.3 ± 1.3*	0.04
	Extensive	4	3.1 ± 1.9	
Stage	2 a	11	2.3 ± 1.9	0.755
	2 b	4	2.5 ± 3.36	
	3 a	6	1.7 ± 1.2	

3.4. Distribution of APE1 Expression Levels in Study Groups

Regarding the mean fold change of normal cases which was 1.5 (cut-off), the high and low expressions of the cases were considered. In a group of cancer patients who received chemotherapy, high expression of APE1 was detected in 20 patients (57.14%) versus low expression in 15 patients (42.86%). The same results were observed in a group of newly diagnosed cancer patients with significant differences (Table 4).

Table 4. Frequency distribution of *APE1* mRNA expression in cancer patients

Gene expression	Cancer with Cis NO (%)	Newly diagnosed NO (%)
High expression	20(57.14)	32(91.43)
Low expression	15(42.86)	3(8.57)
P-value	0.0229 *	0.0001 **

S: Significant difference * (P≤0.05)
HS: Highly Significant difference ** (P≤0.01)

3.5. Evaluation of the Level of Human C-Type Lectin Domain Family 4 Member M in All Study Groups Using the ELISA Method

Regarding figure 1, the mean concentration of CLEC4M (ng/ml) was high in lung cancer patients who received cisplatin (139.52±25.9 ng/ml) and the newly diagnosed group (110.0±38.1 ng/ml), in comparison to the control group (100.32±47.5 ng/ml) with a significant difference *P*<0.001).

3.6. C-Type Lectin Domain Family 4 Member M Concentration Level Regarding the Demographic Characteristics and Histological Types in the Newly Diagnosed Group

Mean level of CLEC4M concentration was high in patients older than 50 years old (334.9±34.3 ng/ml), females (348.7±1.9 ng/ml), patients with squamous cell carcinoma of the skin and large-cell carcinoma (LCC) cancer types (344.6±33.6 and 352.3±38.8 ng/ml, respectively), and patients at stage 1 and 2a (326.3±30.63 and 344.4 ±43.97 ng/ml, respectively). There was no significant difference between the concentration level of CLEC4M and all parameters in demographic characteristics and histological cancer type, as seen in table 5.

3.7. C-Type Lectin Domain Family 4 Member M Concentration Level Regarding the Demographic Characteristics and Histological Types in the Cisplatin Chemotherapy Group

Mean level of CLEC4M concentration was higher in patients older than 50 years old (140.2±25.4 ng/ml), males (141.2±25.13 ng/ml), patients with adenocarcinoma (138.9±22.29 ng/ml), patients with SCLC extensive cancer type (151.8±28.06 ng/ml) regarding the histological types, patients at stage 2b (149.0±28.7 ng/ml), and patients around 3-6 trails showed a high concentration of CLEC4M. No significant difference was observed between the level of CLEC4M regarding demographic characteristics and histological cancer types (Table 6).

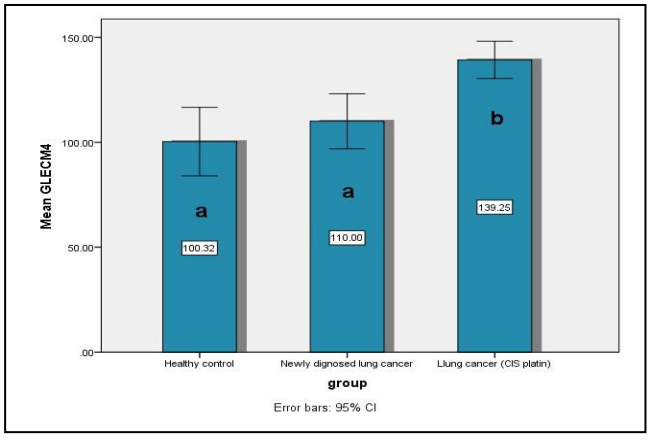

Figure 1. Mean level of CLEC4M (ng/ml) in all study groups

Table 5. CLEC4M concentration associated with demographic parameters and histological type in newly diagnosed lung cancer group

Parameter		N=35	CLEC4M(ng/ml) Mean ± SD	P- value
Age	<50	9	319.7± 48.2	0.31
	>50	26	334.9± 34.3	
Gender	male	33	329.9±39.1	0.507
	female	2	348.7±1.9	
Smoking	smoker	31	333.1 ± 35.5	0.691
	non-smoker	4	322.5 ± 61.4	
Histological type NSCLC SCLC	Adenocarcinoma	19	323.7 ± 38.2a	0.197
	Squamous	7	344.6 ± 33.6	
	LCC	4	352.3 ± 38.8	
	limited	5	320.4	
	extensive	0	0	
Stage	1	13	326.3 ± 30.63	0.692
	2a	11	344.4 ±43.97	
	3b	6	319.4 ± 23.4	
	4a	3	322.5 ± 76.3	
	5a	2	311.0 ± 33.9	

Table 6. CLEC4M concentration associated with demographic parameters and histological type in newly diagnosed lung cancer group

Parameter		Total No.	CLEC4M (ng/ml) concentration	P-value
Age	<50	8	140.2 ± 25.4	0.885
	>50	27	138.9 ± 26.63	
Gender	Male	32	141.2 ± 25.13	0.157
	Female	3	118.8 ± 30.03	
Smoking	Smoker	31	140.2 ± 27.05	0.573
	Non-smoker	4	132.3 ± 14.9	
Histology NSCLC SCLC	Adenocarcinoma	16	138.9 ± 22.29	0.712
	Squamous	6	137.9 ± 35.6	
	LCC	3	125.7 ± 15.58	
	Limited	6	139.6 ± 31.5	0.547
	Extensive	4	151.8 ± 28.06	
Stage	2 a	11	142.6 ± 30.7	0.392
	2 b	4	149.0 ± 28.7	
	3 a	6	138.5 ± 21.6	
	3 b	12	137.7 ± 23.41	
	4 b	2	112.4 ± 25.92	
Drug cycles	1-3	14	137.7 ± 27.39	0.782
	4-6	21	140.26 ± 25.5	

4. Discussion

In the present study, the RT-PCR method was applied to evaluate the APE1 repair gene involved in response to platinum-based chemotherapy in Iraqi lung cancer patients and patients newly diagnosed who were not receiving chemotherapy. The expression of this gene has been investigated in many types of cancers, in which the expression is inversely associated with the survival of patients treated with platinum-based regimens (15-17). Moreover, results of some studies have revealed that high APE1 mRNA levels are associated with platinum-based chemotherapy resistance, while its low levels are correlated with an excellent response to chemotherapy (18, 19).

In the present study, the high mean fold change

expressions of APE1 in the newly diagnosed cancer patients and the cancer patients who received chemotherapy were compared to those of the control population using RT-PCR. The collected data revealed that the mean fold change of the APE1 gene was significantly high in the newly diagnosed cases, compared to the control group, and not related to all demographic characteristics and histopathological types, although stages 3b and 4a had significant mean fold changes.

Results of the present study showed that higher gene expression of the APE1 gene was recorded in the newly diagnosed patients. It is well-accepted that APE1 sub-cellular localization changes are associated with several cellular functions and cancer onset and progression (20, 21). Altered expression of APE1 is often observed in several human tumors (22). Wang et al. (17) found that the APE1 gene expression increased gene expression levels in NSCLC, compared to the SCLC type, specifically in the adenocarcinoma subtype. Gene expression levels correlate positively with stage progress, and these results were expected and obtained previously by another study (16). The polymorphism study of APE1, specially Asp148Glu, suggested that variant genotypes of this gene had a nonsignificant association with an elevated risk of lung cancer carcinogenesis (23).

The results indicated that APE1 gene expression was significantly higher in cancer patients who received cisplatin, compared to the control group. Moreover, there were no significant differences in terms of the demographic characteristics and histopathological types, except for SCLC types, whose APE1 gene expression was significantly higher than that of NSCLC. Expression of APE1 and tumor development was associated with over-expression in many tumors and correlated with the start of chemotherapy resistance (24). Remarkably, down-regulation sensitizes cancer cells to DNA-damaging chemotherapeutic medications and ionizing radiation (25). Many studies have found that APE1 may represent a biomarker for the prediction of prognosis and therapeutic efficacy (17, 26, 27). The leading cause for resistance by the effect of this repair gene in reactive oxygen species is produced by platinum agents (28) and platinum resistance is induced by the reduction of DNA lesions and promotion of redox. Moreover, the promotion of platinum resistance might be induced by Parkin-mediated mitochondrial-specific autophagy in *in vitro* studies (15).

Regarding APE1 fold change levels in cancer patients who received cisplatin, the data were categorized into high and low based on the cutoff value (1.5) of the control group, in which high and low expressions were observed in 57.14% and 42.86% of patients, respectively ($P \leq 0.023$). This result agreed with the findings of a study performed by Zhang et al. (27), who found that the serum APE1 level was significantly elevated in 55.6% of NSCLC patients. The elevated APE1 level in both tissue and serum of patients prior to chemotherapy was associated with worse progression-free survival rates, but not with overall survival rates. After six cycles of chemotherapy, a low APE1 serum level was associated with a better overall survival rate, which means that the APE1 gene is an effective predictive biomarker for chemotherapy (29). Findings of many studies have indicated that APE1 is related to chemotherapeutic outcomes in advanced NSCLC (15, 30, 31).

Results of the present study showed a lower level of expression in the advanced stage four which might be because they were only two cases. Furthermore, nucleotide excision repair (NER) is a major DNA repair mechanism that removes mainly DNA lesions that can distort the DNA helix or form bulky injuries to the genome (32). Therefore, decreased DNA repair within the tumor could cause the decreased removal of platinum-DNA adducts, a significant cause of cell death by this drug, leading to increased clinical response to platinum chemotherapy (30, 33).

In this study, the mean concentration of CLEC4M was highly significant in lung cancer patients who received platinum, compared to the newly diagnosed

lung cancer patients and the control group. However, no significant difference was observed between the two cancer groups in terms of all demographic and histopathological types. Recently, the clinical significance of CLEC4M in cancers has been investigated, and the findings have shown that the high level of CLEC4M in serum might be a potential molecular marker for the diagnosis of early-stage colon cancer (12, 34).

Nonetheless, the biological effects of CLEC4M in lung cancer remain unclear (15, 35). The CLEC4M was correlated significantly with overall survival (OS), and it was developed with an effective prognosis and predictive marker between seven genes in lung squamous cell carcinoma (36). In their study, Tan, Li (12) found that CLEC4M, SLC10A2, and FGF4 genes are involved in lung cancer progression and the regulation of treatment resistance. The CLEC4M is associated with a worse prognosis, and inhibition of CLEC4M showed potential clinical relevance in counterbalancing cisplatin resistance in NSCLC patients.

Recently, Luo, Chen (23) detected that CLEC4M was implicated in the progression of hepatocellular carcinoma, similar to its association with colon and gastric cancer. Notably, patients with high CLEC4M expression levels in their tumor tissues experienced more frequent recurrences and shorter OS time, compared to the low-expression group. This means that this valuable marker correlates with progression and tumourigenesis (23). Results of *in vitro* testing showed that cisplatin treatment combined with CLEC4M knockdown significantly decreased lung cancer cell viability in both A549 and H1299 cell lines. The CLEC4M knockdown significantly enhanced cisplatin-induced cell apoptosis in these cells. These findings may explain that cisplatin treatment significantly increased cleaved caspase-3 expression and CLEC4M knockdown (12).

In addition, other studies have found that the higher expression of CLEC4M had shorter OS and FP in comparison to those with lower CLEC4M expression (14). It means a high level of CLEC4M is associated with poor clinical prognosis in lung cancer patients and enhances the resistance of NSCLC cells to cisplatin. The underlying mechanism included resistance by CLEC4M and DNA repair capacity by upregulating APE1 and ERCC1 repairing gene expression (12).

It is concluded that the APE1 gene expression and CLEC4M could be used successfully in expecting the prognosis of lung cancer patients with and without chemotherapy. High expression of CLEC4M is associated with the enhancement of the resistance of NSCLC cells against cisplatin. However, studies about CLEC4M markers are very few worldwide, and there were no previous reports in Iraq; therefore, further studies are necessary.

Authors' Contribution

Study concept and design: Y. M. I. A. A.
Acquisition of data: S. F. H. A.
Analysis and interpretation of data: M. M. M.
Drafting of the manuscript: M. M. M.
Critical revision of the manuscript for important intellectual content: Y. M. I. A. A.
Statistical analysis: S. F. H. A.
Administrative, technical, and material support: S. F. H. A.

Ethics

This study was approved by and performed under the license obtained from the Ethics Committee of the College of Pharmacy, University of Al-Mustansiriyah, Baghdad, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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References

1. Zaorsky NG, Churilla TM, Egleston BL, Fisher SG, Ridge JA, Horwitz EM, et al. Causes of death among cancer patients. Ann Oncol. 2017;28(2):400-7.
2. Barta JA, Powell CA, Wisnivesky JP. Global Epidemiology of Lung Cancer. Ann Glob Health. 2019;85(1).
3. Tsao AS, Scagliotti GV, Bunn PA, Jr., Carbone DP, Warren GW, Bai C, et al. Scientific Advances in Lung Cancer 2015. J Thorac Oncol. 2016;11(5):613-38.
4. Dasari S, Tchounwou PB. Cisplatin in cancer therapy: molecular mechanisms of action. Eur J Pharmacol. 2014;740:364-78.
5. Zivanovic O, Abramian A, Kullmann M, Fuhrmann C, Coch C, Hoeller T, et al. HIPEC ROC I: a phase I study of cisplatin administered as hyperthermic intraoperative intraperitoneal chemoperfusion followed by postoperative intravenous platinum-based chemotherapy in patients with platinum-sensitive recurrent epithelial ovarian cancer. Int J Cancer. 2015;136(3):699-708.
6. Rocha CRR, Silva MM, Quinet A, Cabral-Neto JB, Menck CFM. DNA repair pathways and cisplatin resistance: an intimate relationship. Clinics (Sao Paulo). 2018;73(1):478.
7. Gridelli C, Aapro M, Ardizzoni A, Balducci L, De Marinis F, Kelly K, et al. Treatment of advanced non-small-cell lung cancer in the elderly: results of an international expert panel. J Clin Oncol. 2005;23(13):3125-37.
8. Sengupta S, Mitra S, Bhakat KK. Dual regulatory roles of human AP-endonuclease (APE1/Ref-1) in CDKN1A/p21 expression. PLoS One. 2013;8(7):e68467.
9. Thakur S, Dhiman M, Tell G, Mantha AK. A review on protein-protein interaction network of APE1/Ref-1 and its associated biological functions. Cell Biochem Funct. 2015;33(3):101-12.
10. Ayyildiz D, Antoniali G, D'Ambrosio C, Mangiapane G, Dalla E, Scaloni A, et al. Architecture of The Human Ape1 Interactome Defines Novel Cancers Signatures. Sci Rep. 2020;10(1):28.
11. Manoel-Caetano FS, Rossi AFT, Calvet de Morais G, Severino FE, Silva AE. Upregulation of the APE1 and H2AX genes and miRNAs involved in DNA damage response and repair in gastric cancer. Genes Dis. 2019;6(2):176-84.
12. Tan LM, Li X, Qiu CF, Zhu T, Hu CP, Yin JY, et al. CLEC4M is associated with poor prognosis and promotes cisplatin resistance in NSCLC patients. J Cancer. 2019;10(25):6374-83.
13. Zhang F, Ren S, Zuo Y. DC-SIGN, DC-SIGNR and LSECtin: C-type lectins for infection. Int Rev Immunol. 2014;33(1):54-66.
14. Fu D, Zhang B, Yang L, Huang S, Xin W. Development of an Immune-Related Risk Signature for Predicting Prognosis in Lung Squamous Cell Carcinoma. Front Genet. 2020;11:978.
15. Li Z, Qing Y, Guan W, Li M, Peng Y, Zhang S, et al. Predictive value of APE1, BRCA1, ERCC1 and TUBB3 expression in patients with advanced non-small cell lung cancer (NSCLC) receiving first-line platinum-paclitaxel chemotherapy. Cancer Chemother Pharmacol. 2014;74(4):777-86.
16. Long K, Gu L, Li L, Zhang Z, Li E, Zhang Y, et al. Small-molecule inhibition of APE1 induces apoptosis, pyroptosis, and necroptosis in non-small cell lung cancer. Cell Death Dis. 2021;12(6):503.
17. Wang X, Yue H, Li S, Guo J, Guan Z, Zhu Z, et al. Genetic Polymorphisms in DNA Repair Gene APE1/Ref-1 and the Risk of Neural Tube Defects in a High-Risk Area of China. Reprod Sci. 2021;28(9):2592-601.
18. Burgess JT, Rose M, Boucher D, Plowman J, Molloy C, Fisher M, et al. The Therapeutic Potential of DNA Damage Repair Pathways and Genomic Stability in Lung Cancer. Front Oncol. 2020;10:1256.
19. Xiong Y, Huang BY, Yin JY. Pharmacogenomics of platinum-based chemotherapy in non-small cell lung cancer: focusing on DNA repair systems. Med Oncol. 2017;34(4):48.
20. Collins AR, Harrington V, Drew J, Melvin R. Nutritional modulation of DNA repair in a human intervention study. Carcinogenesis. 2003;24(3):511-5.
21. Poletto M, Di Loreto C, Marasco D, Poletto E, Puglisi F, Damante G, et al. Acetylation on critical lysine residues of Apurinic/apyrimidinic endonuclease 1 (APE1) in triple negative breast cancers. Biochem Biophys Res Commun. 2012;424(1):34-9.
22. Mio C, Gerratana L, Bolis M, Caponnetto F, Zanello A, Barbina M, et al. BET proteins regulate homologous recombination-mediated DNA repair: BRCAness and implications for cancer therapy. Int J Cancer. 2019;144(4):755-66.
23. Luo L, Chen L, Ke K, Zhao B, Wang L, Zhang C, et al. High expression levels of CLEC4M indicate poor prognosis in patients with hepatocellular carcinoma. Oncol

Lett. 2020;19(3):1711-20.
24. Tell G, Demple B. Base excision DNA repair and cancer. Oncotarget. 2015;6(2):584-5.
25. Pascut D, Sukowati CHC, Antoniali G, Mangiapane G, Burra S, Mascaretti LG, et al. Serum AP-endonuclease 1 (sAPE1) as novel biomarker for hepatocellular carcinoma. Oncotarget. 2019;10(3):383-94.
26. Malfatti MC, Gerratana L, Dalla E, Isola M, Damante G, Di Loreto C, et al. APE1 and NPM1 protect cancer cells from platinum compounds cytotoxicity and their expression pattern has a prognostic value in TNBC. J Exp Clin Cancer Res. 2019;38(1):309.
27. Zhang S, He L, Dai N, Guan W, Shan J, Yang X, et al. Serum APE1 as a predictive marker for platinum-based chemotherapy of non-small cell lung cancer patients. Oncotarget. 2016;7(47):77482-94.
28. Kelley MR, Jiang Y, Guo C, Reed A, Meng H, Vasko MR. Role of the DNA base excision repair protein, APE1 in cisplatin, oxaliplatin, or carboplatin induced sensory neuropathy. PLoS One. 2014;9(9):e106485.
29. Zhang H, Ba S, Yang Z, Wang T, Lee JY, Li T, et al. Graphene Quantum Dot-Based Nanocomposites for Diagnosing Cancer Biomarker APE1 in Living Cells. ACS Appl Mater Interfaces. 2020;12(12):13634-43.
30. Chen SH, Chang JY. New Insights into Mechanisms of Cisplatin Resistance: From Tumor Cell to Microenvironment. Int J Mol Sci. 2019;20(17).
31. Yang X, Peng Y, Jiang X, Lu X, Duan W, Zhang S, et al. The regulatory role of APE1 in epithelial-to-mesenchymal transition and in determining EGFR-TKI responsiveness in non-small-cell lung cancer. Cancer Med. 2018;7(9):4406-19.
32. Rouillon C, White MF. The evolution and mechanisms of nucleotide excision repair proteins. Res Microbiol. 2011;162(1):19-26.
33. Acklin S, Xia F. The Role of Nucleotide Excision Repair in Cisplatin-Induced Peripheral Neuropathy: Mechanism, Prevention, and Treatment. Int J Mol Sci. 2021;22(4).
34. Budisan L, Gulei D, Jurj A, Braicu C, Zanoaga O, Cojocneanu R, et al. Inhibitory Effect of CAPE and Kaempferol in Colon Cancer Cell Lines-Possible Implications in New Therapeutic Strategies. Int J Mol Sci. 2019;20(5).
35. Yu Q, Gao K. CLEC4M overexpression inhibits progression and is associated with a favorable prognosis in hepatocellular carcinoma. Mol Med Rep. 2020;22(3):2245-52.
36. Ballatori N, Krance SM, Notenboom S, Shi S, Tieu K, Hammond CL. Glutathione dysregulation and the etiology and progression of human diseases. Biol Chem. 2009;390(3):191-214.