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Roles of LCN2, PDIA3 and HGF in Progression and Remission of Non-small Cell Lung Cancer

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KEYWORDS Hepatocyte Growth Factor. Lipid-carrying Protein 2. Non-small Cell Lung Cancer. Protein Disulfide-isomerase a3

ABSTRACT The research aimed to investigate the expressions of lipid-carrying protein 2 (LCN2), protein disulfide-isomerase A3 (PDIA3) and hepatocyte growth factor (HGF) during the progression of non-small cell lung cancer (NSCLC), and their predictive values for disease remission. A total of 120 NSCLC patients admitted from January 2021 to January 2022 were enrolled. The patients with complete remission and partial remission were assigned into the remission group, while those with stable disease and progressive disease were assigned into the non-remission group. LCN2, PDIA3 and HGF had higher positive expression rates in the case of tumour diameter more than or equal to 3 cm, TNM stage 3/4, moderate and low differentiation, lymph node metastasis and deep invasion ($P<0.05$). The predictive efficiency of combination of LCN2, PDIA3 and HGF for disease remission was highest. There were positive correlations among LCN2, PDIA3 and HGF ($P<0.05$). LCN2, PDIA3 and HGF are highly expressed in NSCLC, and their expressions are elevated with disease progression.

INTRODUCTION

Non-small cell lung cancer (NSCLC), as a common disease in clinical practice, has been treated by various methods and drugs (Yuan et al. 2019). Nevertheless, the remission rate of some patients remains low, which influences their survival rate (Duma et al. 2019). NSCLC progression has been associated with many factors, among which cytokines have been mostly investigated (Misra and Singh 2019).

Lipid-carrying protein 2 (LCN2), as a type of glycoprotein, is capable of transporting hydrophobic ligands across cell membranes, modulating immune responses, maintaining iron homeostasis and facilitating epithelial cell differentiation (Chi et al. 2020; Bahrun et al. 2021; Olson et al. 2021). In the normal tissues of the human body, LNC2 has low or no expression. However, once malignant lesions form, the level of LCN2 increases sharply (Santiago-Sánchez et al. 2020). Therefore, LCN2 has become a potential biomarker for human malignant tumours (Xu et al. 2020).

Protein disulfide-isomerase A3 (PDIA3), as a member of the protein disulfide isomerase family, mainly exists in the endoplasmic reticulum of most cells (Lam and Lim 2021). Consisting of 505 amino acids, human PDIA3 gene is located on the chromosome 15q15.3 (Song et al. 2021). PDIA3 can induce oxidative damage and participates in endoplasmic reticulum stress, ultimately facilitating the tumorigenic transformation of tumour-related oxidation-dependent regulatory factors, and inducing malignant tumours (Chiavari et al. 2020). It is well-documented that PDIA3 is capable of regulating platelet function, taking part in antigen presentation, modulating immunity and participating in the onset and progression of malignant tumours (Hu et al. 2019; Chiavari et al. 2020; Diaz et al. 2021; Zhang et al. 2020). Moreover, hepatocyte growth factor (HGF), as a polypeptide growth factor, participates in the onset and progression of various malignant tumours by specifically binding its receptor protein c-MET (Raghav et al. 2012). After the binding, the tyrosine residues in cells are phosphorylated, which activates tyrosine kinases in the cyto-

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plasm (Liu et al. 2017; Titmarsh et al. 2020). Then many effector proteins in the cytoplasm recruit phosphorylated carboxyl terminals and are rapidly phosphorylated, thus activating signal transducer and activator of transcription 3 (STAT3), mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K) and other signal transduction pathways, and ultimately participating in various physiological and pathological processes (Moosavi et al. 2019). Recently, HGF has also been involved in NSCLC progression (Liang and Wang 2020).

Objectives

Variations in the expressions of LCN2, PDIA3 and HGF during NSCLC progression were analysed and their values for predicting disease remission were evaluated, aiming to identify the effective indicators for predicting the remission of NSCLC, and to provide references for clinical research.

MATERIAL AND METHODS

Subjects

A total of 120 NSCLC patients admitted to the researchers' hospital from January 2021 to January 2022 were enrolled according to the inclusion and exclusion criteria below. The subjects consisted of 65 males and 55 females aged 40-65 years old, (50.42±6.87) years on average. There were 69 cases with tumour diameter more than or equal to 3 cm, and 51 cases less than 3 cm. Among them, 56 patients were in stage 1/2, and 64 were in stage 3/4. Lymph node metastasis was found in 43 patients. NSCLC was moderately and lowly differentiated in 67 cases, but highly differentiated in 53 cases. Besides, 89 cases had superficial invasion, and 31 cases had deep invasion. Meanwhile, 67 patients had squamous cell carcinoma, and 53 had adenocarcinoma. This study was reviewed and approved by the ethics committee of the hospital, and informed consent was obtained from the patients and their family members.

The inclusion criteria involved patients meeting the diagnostic criteria for NSCLC proposed by NCCN (Ettinger et al. 2021), and with the diagnosis confirmed by cytology, pathology and imaging, those voluntarily participating in this study, and those who had not undergone radiotherapy or chemotherapy.

The exclusion criteria were patients complicated with other tumours or non-primary lung cancer, those with congenital immune disorders, those with organ dysfunction (heart, kidney, etc.), those with mental disorders, or those unable to communicate.

Collection of Tissues

Cancer and paracancerous tissues surgically resected were collected, fixed with paraformaldehyde, embedded in paraffin and sectioned for later use.

Detection of LCN2, PDIA3 and HGF Expressions by Immunohistochemistry

The paraffin sections were deparaffinized, hydrated with gradient concentrations of ethanol solutions, added protease repair solution and incubated for 0.5 hours. Afterwards, the repair solution was aspirated, and the tissues were transferred into a wet box, blocked, and incubated for 0.5 hours. Later, the blocking buffer was aspirated, and the primary antibodies against LCN2, PDIA3 and HGF (Abcam, USA) diluted at 1:400 were added to the tissues, followed by incubation with secondary antibodies (Abcam, USA) at room temperature. Sixty minutes later, the tissues were taken out, washed with PBS, stained with DAB, dehydrated with gradient concentrations of ethanol solutions and rinsed with xylene. Finally, they were mounted, and cell staining results were observed. The expressions of indicators were determined according to the degree of cell staining (brownish yellow = 3 points, yellow = 2 points, pale yellow = 1 point and unstained = 0 point) and the proportion of stained cells (>51% = 3 points, 26-50% = 2 points, 6-25% = 1 point and <5% = 0 point). A score of $\geq$2 points indicated positive expression.

Evaluation of Treatment Outcomes

The treatment outcomes were evaluated according to the Response Evaluation Criteria in Solid Tumours, which included complete remission (CR), partial remission (PR), stable disease (SD) and progressive disease (PD). CR was defined as disappearance of all target lesions, PR as reduction of sum of the maximum diameter of lesions more than or equal to 30 percent, SD as decrease of sum of the maximum diameter of lesions less than 30 percent or increase of sum of the maximum diameter of

lesions less than 20 percent, and PD as increase of sum of the maximum diameter of lesions more than or equal to 20 percent or formation of new lesion. According to the remission status, the subjects were assigned into the remission group (CR + PR) and non-remission group (SD + PD).

Statistical Analysis

SPSS 22.0 software was utilised for statistical analysis. The count data were expressed as frequency and percentage, and compared by the χ^2 test between groups. Pearson's correlation analysis was conducted. Logistic regression model was employed to analyse the influencing factors, and the values of LCN2, PDIA3 and HGF for predicting disease remission in NSCLC were analysed by receiver operator characteristic (ROC) curves. P<0.05 indicated statistically significant differences.

RESULTS

Expressions of LCN2, PDIA3 and HGF in NSCLC and Paracancerous Tissues

The positive expression rates of LCN2, PDIA3 and HGF in NSCLC tissues were significantly higher than those in paracancerous tissues (P<0.05) (Table 1 and Fig. 1).

Expression Changes of LCN2, PDIA3 and HGF During NSCLC Progression

LCN2, PDIA3 and HGF were involved in NSCLC progression, and their expressions varied in the cases of different tumour diameters, TNM stage, differentiation degree, lymph node metastasis status and depth of invasion. The positive expression rates of LCN2, PDIA3 and HGF were higher in the case of tumour diameter more than or equal to 3 cm, TNM stage 3/4, moderate and low differentiation, presence of lymph node metastasis and deep invasion (P<0.05) (Table 2).

Baseline Data and Expressions of LCN2, PDIA3 and HGF in Remission and Non-remission Groups

No significant differences were found in the sex ratio and age between remission and non-remission groups (P>0.05). Significant differences were observed in tumour diameter, TNM stage, differentiation degree, lymph node metastasis status, depth of invasion, and expressions of LCN2, PDIA3 and HGF between the two groups (P<0.05).

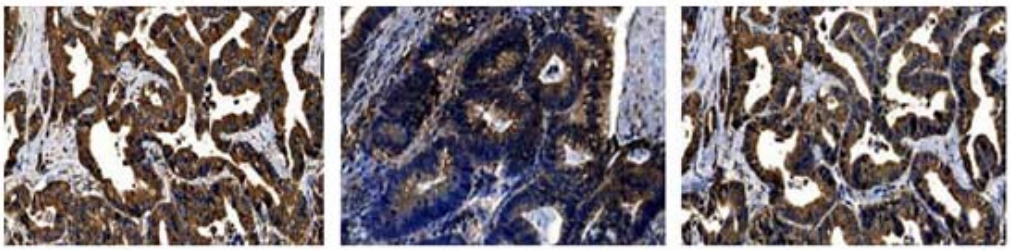

Fig. 1. Positive expressions of LCN2, PDIA3 and HGF. HGF: Hepatocyte growth factor; LCN2: lipid-carrying protein 2; PDIA3: protein disulfide-isomerase A3

Table 1: Expressions of LCN2, PDIA3 and HGF in NSCLC and paracancerous tissues (n, %)

	n	*LCN2*	*PDIA3*	*HGF*
Paracancerous tissue	120	11 (9.17)	14 (11.67)	10 (8.33)
Cancer tissue	120	76 (63.33)	89 (74.17)	91 (75.83)
x^2		76.178	95.670	112.162
P		<0.001	<0.001	<0.001

HGF: Hepatocyte growth factor; LCN2: lipid-carrying protein 2; NSCLC: non-small cell lung cancer; PDIA3: protein disulfide-isomerase

Table 2: Expression changes of LCN2, PDIA3 and HGF during NSCLC progression (n, %)

		n	*LCN2*	χ^2/P	*PDIA3*	χ^2/P	*HGF*	χ^2/P
Tumor diameter (cm)	>3 cm	69	50 (72.46)	5.828/<0.001	59 (85.51)	10.898/<0.001	60 (86.96)	10.961/<0.001
	<3 cm	51	26 (50.98)		30 (58.82)		31 (60.78)	
TNM stage	1/2	56	27 (48.21)	10.336/<0.001	34 (60.71)	9.917/<0.001	33 (58.93)	16.373/<0.001
	3/4	64	49 (76.56)		55 (85.94)		58 (90.63)	
Differentiation degree	Moderate and low differentiation	67	60 (89.55)	44.906/<0.001	59 (88.06)	15.282/<0.001	62 (92.54)	23.096/<0.001
	High differentiation	53	16 (30.19)		30 (56.60)		29 (54.72)	
Lymph node metastasis	Yes	43	39 (90.70)	21.609/<0.001	39 (90.70)	9.558/<0.001	38 (88.37)	5.749/<0.001
	No	77	37 (48.05)		50 (64.94)		53 (68.83)	
Depth of invasion	Superficial	89	49 (55.06)	10.164/<0.001	60 (67.42)	8.195/<0.001	61 (68.54)	10.002/<0.001
	Deep	31	27 (87.10)		29 (93.55)		30 (96.77)	

HGF: Hepatocyte growth factor; LCN2: lipid-carrying protein 2; NSCLC: non-small cell lung cancer; PDIA3: protein disulfide-isomerase A3

The non-remission group had significantly higher positive expression rates of LCN2, PDIA3 and HGF than those of the remission group (P<0.05) (Table 3).

Analysis Results of Factors Affecting Disease Remission of NSCLC Patients

Using disease remission as the dependent variable (remission = 0, non-remission = 1) and tumour diameter, TNM stage, differentiation degree, lymph node metastasis status, depth of invasion and expressions of LCN2, PDIA3 and HGF as independent variables, a logistic regression model was established. The results indicated that high expressions of LCN2, PDIA3 and HGF were risk factors affecting the disease remission of NSCLC patients in addition to progression (Table 4).

Predictive Values of LCN2, PDIA3 and HGF for Disease Remission of NSCLC Patients

ROC curve analysis revealed that the combination of LCN2, PDIA3 and HGF for predicting the disease remission of patients had higher predictive efficiency than those of LCN2, PDIA3 and HGF alone. The area under the curve of LCN2, PDIA3 and HGF in combination for predicting disease remission was 0.934 (95% CI: 0.885-1.231), and the sensitivity and specificity were 90.00 percent and 84.50 percent, respectively (P<0.001) (Fig. 2).

DISCUSSION

High LCN2 expression can facilitate the proliferation of malignant cells, angiogenesis and cell invasion (Rahimi et al. 2020; Villodre et al. 2021). LCN2 is highly expressed in the serum of lung cancer patients, and associated with pathological differentiation, staging and lymph node metastasis, and it is a potential marker for the clinical diagnosis of lung cancer due to its role in disease progression through modulating the degradation of matrix metalloproteinase-9 (MMP-9) (Fan et al. 2021). Likewise, in this study, high LCN2 expression was correlated with tumour diameter, TNM stage, differentiation degree, lymph node metastasis and depth of invasion of NSCLC, and its high expression was further enhanced during disease progression. Besides, the patients without disease remission had higher LCN2 expression than that of the patients

Table 3: Baseline data and expressions of LCN2, PDIA3 and HGF in remission and non-remission groups

		Remission group (n=81)	*Non-remission group (n=39)*	*Statistical value*	*P*
Sex	Male	45 (55.56)	20 (51.28)	0.194	0.660
	Female	36 (44.44)	19 (48.72)		
Age (year)	51.32±6.19	51.36±6.23	0.033	0.974	
Tumor diameter (cm)	≥3 cm	40 (49.38)	29 (74.36)	6.720	0.010
	<3 cm	41 (50.62)	10 (25.64)		
TNM stage	1/2	47 (58.02)	9 (23.08)	12.918	0.001
	3/4	34 (41.98)	30 (76.92)		
Differentiation degree	Moderate and low differentiation	24 (29.63)	34 (87.18)	29.399	0.001
	High differentiation	48 (59.26)	5 (12.82)		
Lymph node metastasis	Yes	11 (13.58)	32 (82.05)	53.677	0.001
	No	70 (86.42)	7 (17.95)		
Depth of invasion	Superficial	76 (93.83)	13 (33.33)	50.281	0.001
	Deep	5 (6.17)	26 (66.67)		
LCN2		46 (56.79)	30 (76.92)	4.595	0.032
PDIA3		53 (65.43)	36 (92.31)	9.924	0.001
HGF		56 (69.14)	35 (89.74)	6.100	0.014

HGF: Hepatocyte growth factor; LCN2: lipid-carrying protein 2; NSCLC: non-small cell lung cancer; PDIA3: protein disulfide-isomerase A3

Table 4: Analysis results of factors affecting disease remission of NSCLC patients

Variable	*Regression coefficient*	*Standard error*	*Wald x^2*	*P*	*OR*	*95% CI*
Constant	-26.227	5.847	13.525	0.000	-	-
LCN2	0.884	0.546	10.924	0.000	1.665	1.078~4.399
PDIA3	0.728	0.575	9.321	0.000	1.453	0.723~3.756
HGF	0.633	0.598	8.112	0.000	1.218	0.703~3.544

CI: Confidence interval; HGF: hepatocyte growth factor; LCN2: lipid-carrying protein 2; NSCLC: non-small cell lung cancer; OR: odds ratio; PDIA3: protein disulfide-isomerase A3

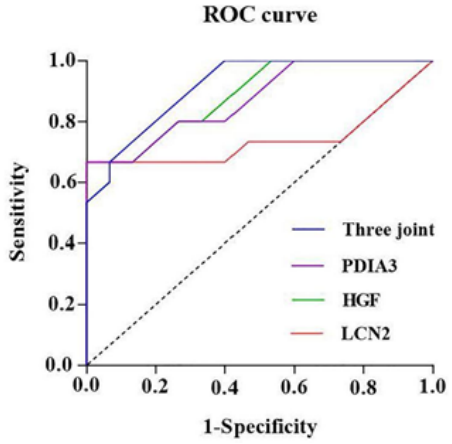

Fig. 2. ROC curves of LCN2, PDIA3 and HGF for predicting disease remission in NSCLC patients. HGF: Hepatocyte growth factor; LCN2: lipid-carrying protein 2; NSCLC: non-small cell lung cancer; PDIA3: protein disulfide-isomerase A3; ROC: receiver operator characteristic

with disease remission, indicating an association between LCN2 and disease remission. Probably, LCN2 expression rises gradually during disease progression, leading to aberrant MMP-9 degradation. Since the disease deteriorates in patients who do not respond to treatment, LCN2 expression further increases.

By facilitating the proliferation of cancer cells, high PDIA3 expression accelerates further deterioration of malignant tumours in a short period of time (Zhang et al. 2022). In this study, PDIA3 expression was elevated in NSCLC tissues, and it rose sharply during disease progression, being in line with the results of Wang et al. (2017). Moreover, the researchers found that PDIA3 was an influencing factor for the disease remission of NSCLC patients, as a potential predictor.

After HGF specifically binds c-MET, it can repair tissue injury and boost embryonic development under normal conditions, but activate STAT3,

MAPK and PI3K signal transduction pathways to induce cancerization in the pathological state (Cascone et al. 2017; Yin et al. 2019). In this study, HGF rose gradually during NSCLC progression, indicating that HGF was involved in this process. Possibly, related pathways are activated by HGF, promoting the invasion of cancer cells. The researchers found that high HGF expression was able to predict the disease remission of NSCLC patients. Therefore, HGF is applicable to the early prediction of disease remission, and targeted interventions can be formulated to improve the prognosis of patients.

CONCLUSION

In conclusion, LCN2, PDIA3 and HGF are highly expressed in NSCLC patients, and their expressions increase along with disease progression and are associated with disease remission. Therefore, they can be utilised for the early prediction of disease remission.

RECOMMENDATIONS

Further animal and clinical studies are still in need to further validate the findings of this study.

ABBREVIATIONS

- CR: Complete Remission
- HGF: Hepatocyte Growth Factor
- LCN2: Lipid-Carrying Protein 2
- MAPK: Mitogen-Activated Protein Kinase
- MMP-9: Matrix Metalloproteinase-9
- NSCLC: Non-Small Cell Lung Cancer
- PD: Progressive Disease
- PDIA3: Protein Disulfide-Isomerase A3
- PI3K: Phosphatidylinositol 3-kinase
- PR: Partial Remission
- ROC: Receiver Operator Characteristic
- SD: Stable Disease
- STAT3: Signal Transducer and Activator of Transcription 3

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