Upregulation of glucosamine-phosphate N-acetyltransferase 1 is a promising diagnostic and predictive indicator for poor survival in patients with lung adenocarcinoma

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Abstract. Lung adenocarcinoma, a type of non-small cell lung cancer, is the leading cause of cancer death worldwide. Great efforts have been made to identify the underlying mechanism of adenocarcinoma, especially in relation to oncogenes. The present study by integrating computational analysis with western blotting, aimed to understand the role of the upregulation of glucosamine-phosphate N-acetyltransferase 1 (GNPNAT1) in carcinogenesis. In the present study, publicly available gene expression profiles and clinical data were downloaded from The Cancer Genome Atlas to determine the role of GNPNAT1 in lung adenocarcinoma (LUAD). In addition, the association between LUAD susceptibility and GNPNAT1 upregulation were analyzed using Wilcoxon signed-rank test and logistic regression analysis. In LUAD, GNPNAT1 upregulation was significantly associated with disease stage [odds ratio (OR)=2.92, stage III vs. stage I], vital status (dead vs. alive, OR=1.89), cancer status (tumor status vs. tumor-free status, OR=1.85) and N classification (yes vs. no, OR=1.75). Cox regression analysis and the Kaplan-Meier method were utilized to evaluate the association between GNPNAT1 expression and overall survival (OS) time in patients with LUAD. The results demonstrated that patients with increased GNPNAT1 expression levels exhibited a reduced survival rate compared with those with decreased expression levels ($P=8.9x10^{-5}$). In addition, Cox regression analysis revealed that GNPNAT1 upregulation was significantly associated with poor OS time [hazard ratio (HR): 1.07; 95% confidence interval (CI): 1.04-1.10; P<0.001]. The gene set enrichment analysis revealed that 'cell cycle', 'oocyte meiosis', 'pyrimidine mediated metabolism', 'ubiquitin mediated proteolysis', 'one carbon pool by folate', 'mismatch repair progesterone-mediated oocyte maturation' and 'basal transcription factors purine metabolism' were differentially enriched in the GNPNAT1 high-expression samples compared with GNPNAT1 low-expression samples. The aforementioned pathways are involved in the pathogenesis of LUAD. The findings of the present study suggested that GNPNAT1 upregulation may be considered as a promising diagnostic and prognostic biomarker in patients with LUAD. In addition, the aforementioned pathways may be pivotal pathways perturbed by the abnormal expression of GNPNAT1 in LUAD. The findings of the present study demonstrated the therapeutic value of the regulation of GNPNAT1 in lung adenocarcinoma.

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Introduction

Lung cancer is a deadly disease with an incidence rate of 11.4% worldwide in 2020 (1-4). The pathogenesis of lung cancer is associated with genetic and epigenetic factors, such as MYC amplification, deregulated expression and epigenetic inactivation of Ras Association Domain Family 1 (5-8). In the USA, lung cancer is the leading cause of cancer-related deaths in both men and women, with a mortality rate of 12.7%. This disease is an aggressive type of cancer, with a 5-year overall survival rate of 14% for advanced stage disease (3). There are two major types of lung cancer, namely small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) (9). The former one is responsible for ~15% of all lung cancer cases (10). SCLC tumors tend to be more aggressive and may be not diagnosed until they have already metastasized (11). NSCLC is the most common subtype of lung cancer, being responsible for ~85% of lung cancer cases (12). Among the different subtypes of NSCLC, lung adenocarcinoma (LUAD) is the most common type, accounting for >50% of NSCLC cases with an increasing incidence rate (13).

The 5-year overall survival rate for stage I SCLC and NSCLC is 50 and 60-70%, respectively (14,15). However, the survival rate in patients with advanced lung cancer is almost 15% (12). In total, ~70% of patients with lung cancer present with advanced stage of the disease at the time of diagnosis (12), supporting the lack of efficient methods for early diagnosis. The early signs of lung cancer are usually subtle or non-specific, such as cough, irritating dry cough or choking cough (16). In clinical practice, spiral computed tomography and fluorescence bronchoscopy are commonly used to detect tumors with a size of >1 mm (17,18). However, this resolution is commonly insufficient to diagnose stage I lung cancer (19). Hence, the diagnosis of patients at a very early stage of the disease and the massive screening of individuals at increased risk of developing lung cancer warrants the need to investigate the genetic basis of carcinogenesis. In terms of DNA mutations, point mutations in the KRAS gene (20), frame shift deletions or insertions in *TP53* (21), and microsatellite alterations may trigger the occurrence of lung cancer (22). In addition, at the transcriptional level, hypermethylated gene promoters may serve as biomarkers for the early detection of lung cancer (23). A study demonstrated that cyclin-dependent kinase inhibitor 2A (CDKN2A), which is involved in the cell cycle, and O6-methylguanine DNA methyltransferase, which is involved in DNA repair, were both downregulated in lung cancer samples compared with paracarcinoma tissue (24).

Glucosamine-phosphate N-acetyltransferase 1 (GNPNAT1) encodes an enzyme involved in the pathway mediating the biosynthesis of uridine diphosphate N-acetylglucosamine (UDP-GlcNAc), an important donor substrate for N-linked glycosylation, which is in turn involved in metabolism in eukaryotic cells (25). For example, silencing of GNPNAT1 in pancreatic β-cells modulated insulin secretion, while its increased methylation status was associated with reduced risk of developing type 2 diabetes mellitus (26). It has been reported that metabolism-related genes serve crucial roles in tumor progression (27). The levels of UDP-GlcNAc are known to affect hyaluronan synthesis and protein O-GlcNAcylation (28). Growing evidence has suggested that O-GlcNAcylation promotes cell survival via the aberrant metabolic state of malignant tumors (29).

GNPNAT1 serves as a biomarker for predicting prostate and colorectal cancer biochemical recurrence (30). A study demonstrated that the levels of lactate dehydrogenase A, lysophosphatidylglycerol acyltransferase 1, GNPNAT1, prostaglandin E synthase and thymidylate synthase were increased in lung cancer tissues compared with para-carcinoma tissues (31). However, the association between GNPNAT1 expression and the early diagnosis and prognosis of LUAD has not been fully investigated.

Hence, the present study aimed to comprehensively evaluate the diagnostic and prognostic potential of GNPNAT1 expression in human LUAD based on publicly available data from The Cancer Genome Atlas (TCGA). In addition, gene set enrichment analysis (GSEA) was carried out to identify the biological pathways involved in LUAD, perturbated by the GNPNAT1 regulatory network. The changes in the protein expression levels of GNPNAT1 in patients with LUAD were validated by western blotting. The findings of the present study demonstrated the therapeutic value of the regulation of GNPNAT1 in lung adenocarcinoma.

Materials and methods

Gene expression data and bioinformatics analysis. Gene expression data and corresponding clinical information of 585 individuals were downloaded from the TCGA official website (https://portal.gdc.cancer.gov; Project ID: TCGA-LUAD (32,33). Hence, the clinical data of 522 patients are shown in Table I. In addition, 63 healthy subjects were also included in this study. Also, the differential expression of GNPNAT1 between tumor and paracarcinoma tissues was conducted. The majority of the patients (81.0%) were >55 years and 53.6% were female. Boxplots and dot plots were used to visualize the differences in gene expression among different groups analyzed using the downloaded data (34). The classification systems was based on the Tumor Node Metastasis (TNM) classification of malignant tumors, 5th edition, 1997 (35).

GSEA. GSEA (v.4.1.0; http://www.gsea-msigdb.org/gsea/index.jsp) as performed as previously described (36). GSEA was performed to reveal the significant survival differences between the high [FPKM (fragments per kilo- base of transcript per million mapped reads) ≥ 10] and low GNPNAT1 (FPKM<10) expression groups. All genes were then ranked according to their association with the GNPNAT1 high-expression phenotype. The nominal (NOM) P-value and normalized enrichment score (NES) were utilized to sort the pathways enriched in each phenotype (36,37). In GSEA, pathways showing NOM P-value ≤0.05 or false discovery rate (FDR) q-value ≤0.05 were considered as significant (38).

Western blotting. Frozen tissues of 35 patients, those without any other diagnosed type of cancers, including 9 males and 26 females, age range, 32-77 years (median age, 60 years) were used to perform western blotting. All patients were recruited to the Department of Thoracic and Cardiovascular Surgery, The Second Affiliated Hospital of Nantong University (Nantong, China) during April of 2020. The present study was approved by the Ethics Committee of The Second Affiliated Hospital of Nantong University and in compliance with the Declaration of Helsinki. Written patient consent for use of their tissues in research was obtained. After surgical resection of the tissue, the central non-necrotic area was taken as the cancer tissue sample with sterile scissors, and then adjacent tissue 2-3 cm away from the edge of the cancer was obtained as the paracarcinoma tissue with another new sterile scissors and the remaining part was sent for biopsy. The frozen tissue samples were pulverized under -80˚C using a mortar and pestle to extract proteins. Briefly, the cultured cells were rinsed thrice with precooled PBS. Subsequently, the cells were lysed with RIPA buffer (cat. no. P0013K) supplemented with phenylmethanesulfonylfluoride (PMSF; cat. no. ST506; both from Beyotime Institute of Biotechnology) at 4˚C for 30 min and centrifuged at 14,000 x g at 4˚C for 30 min. The supernatant containing the protein extracts was collected and proteins were quantified using the BCA Protein Assay kit (Thermo Fisher Scientific, Inc.). Proteins (50 μg/lane) were separated by 8% SDS-PAGE (cat. no. ab139604; Abcam), and

Table I. Characteristics of patients with LUAD (n=522) according to datasets from The Cancer Genome Atlas.

Clinical characteristics	No. of patients[a]	%[b]
Age at diagnosis, years		
≤55	80	15.3
>55	423	81.0
Stage		
I	279	53.4
II	124	23.8
III	85	16.3
IV	26	5.0
Sex		
Male	242	46.4
Female	280	53.6
T classification		
T1	172	33.0
T2	281	53.8
T3	47	9.0
T4	19	3.6
N classification		
N0	335	64.2
N1	98	18.8
N2	75	14.4
N3	2	0.4
M classification		
M0	353	67.6
M1	25	4.8
Recurrence		
No	279	53.4
Yes	146	28.0
Survival status		
Death	188	36.0
Survival	334	64.0
Neoplasm cancer status		
With tumor	141	27.0
Tumor free	248	47.6
Histological type		
Acinar cell neoplasms	22	4.2
Adenomas and adenocarcinomas	486	93.1
Cystic, mucinous and serous neoplasms	14	2.7
Radiation therapy		
No	377	72.2
Yes	58	11.1
Cigarette history		
No	166	31.8
Yes	356	68.2

[a]The sum of all the numbers in each category is not always 522, because not everyone has every piece of diagnostic information. [b]The percentage is calculated according to the proportion of the actual number of the category in the total number of 522 people. T, tumor; N, node; M, metastasis; LUAD, lung adenocarcinoma.

then transferred onto polyvinylidene difluoride membranes (EMD Millipore). Following blocking with 5% skimmed milk dissolved in Tris-buffered saline Tween-20 (0.1% TBST) for 1 h at room temperature, the membranes were rinsed with 0.1% TBST thrice. Subsequently, the membranes were incubated with primary antibodies at 4˚C overnight. The following primary antibodies were used: Anti-GNPNAT1 polyclonal antibody (1:1,0000; cat. no. K107882P) and anti-GAPDH polyclonal antibody (1:1,0000, cat. no. K106389P; both from Beijing Solarbio Science & Technology Co., Ltd.). The next day, the membranes were incubated with the corresponding HRP-conjugated mouse anti-Human IgG1 FC secondary antibody (1:1,0000 dilution cat. no. AS16-3223; Agrisera AB). The immunoreactive bands were visualized using a developing and fixing kit (P0020-1; Beyotime Institute of Biotechnology). GAPDH was used as the loading control.

Statistical analysis. R statistical software v.3.5.3 (39) was used to perform the statistical analyses. The association between cancer susceptibility and the GNPNAT1 high-expression phenotype was evaluated using both the Wilcoxon signed-rank test and logistic regression. Cox regression analysis and the Kaplan-Meier method were performed to determine the association between GNPNAT1 expression and overall survival (OS) time in patients with LUAD. Multivariate Cox analysis was conducted to evaluate the association between the expression of GNPNAT1 and other clinical features, such as clinical stage, sex, tumor (T) classification, node (N) classification, metastasis (M) classification, recurrence, survival status, cancer status, histological type, radiation therapy and smoking status. Tests among multiple groups of samples, such as advanced clinical stage (stage I vs. stage II vs. stage III vs. stage IV), were assayed by Kruskal Wallis test and the post hoc Dunn's test. In the survival analysis, the cut-off value of GNPNAT1 expression was set to 10. $P<0.05$ was considered to indicate a statistically significant difference and $P<0.01$ was considered to indicate a highly statistically significant difference.

Results

Patient characteristics. As shown in Table I, the clinical and gene expression data of 522 patients were obtained from TCGA. The majority of the patients (81.0%) were >55 years. A total of 279 patients (53.4%) were of stage I, 124 (23.8%) of stage II, 85 (16.3%) of stage III and 26 (5.0%) of stage IV. The majority of tumor samples (93.1%; n=486) were classified as adenomas and adenocarcinomas, which were significant standard clinical indexes. Among the 522 patients, 146 (28.0%) experienced cancer recurrence. Regarding cancer status, 248 patients (47.6%) were of tumor-free status and 141 of tumor status (27.0%), while 11.1% (n=58) underwent intensity-modulated radiation therapy. Finally, 68.2% of all participants had history of cigarette smoking, supporting the strong association between smoking and LUAD.

GNPNAT1 expression increases in LUAD. The expression data of GNPNAT1 from 522 samples were obtained from TCGA. As shown in Fig. 1, GNPNAT1 was differentially expressed between normal and tumor samples (Fig. 1A; normal vs. tumor

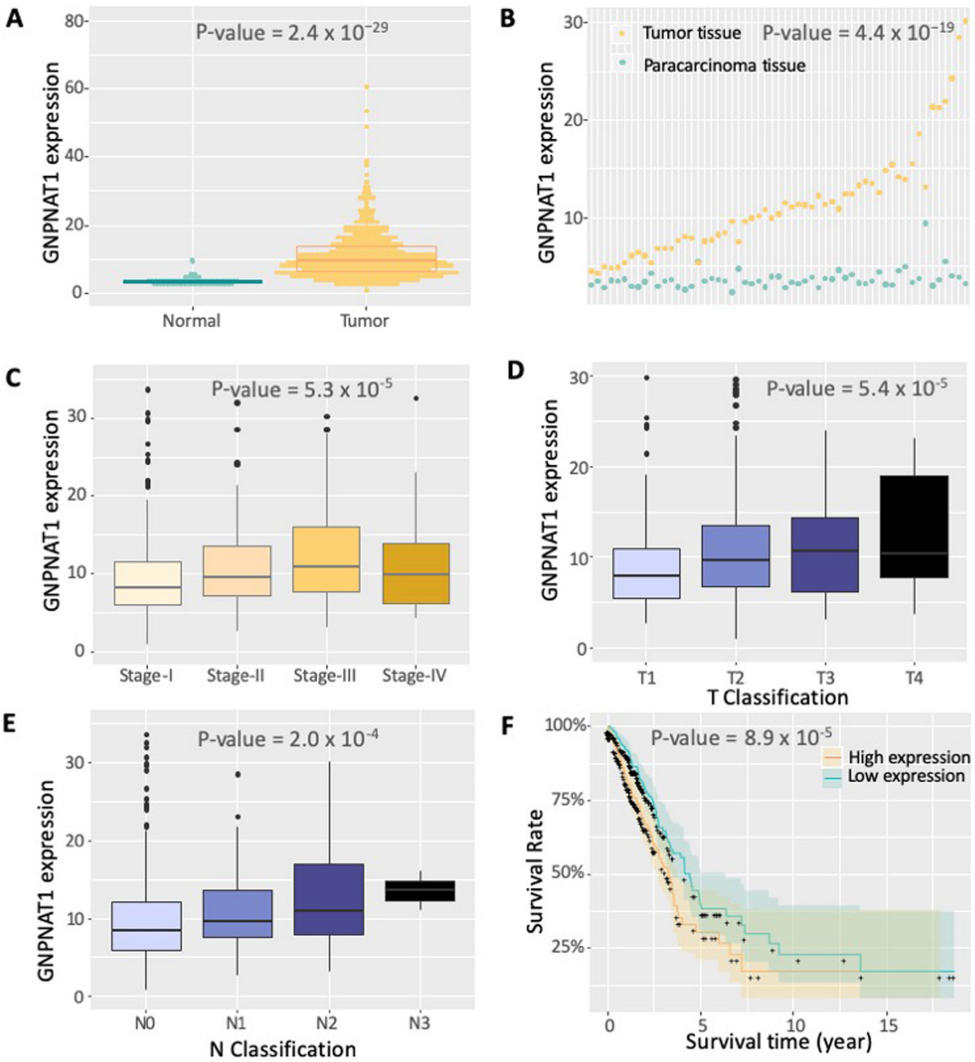

Figure 1. GNPNAT1 expression in LUAD from The Cancer Genome Atlas. (A) Expression levels of GNPNAT1 in the same anatomical sites of the lung tissue between normal subjects and patients with LUAD are shown. (B) Expression levels of GNPNAT1 in tumor and para-carcinoma tissues isolated from the same patient are shown. The data in each column are paired from the same individual. (C) Expression levels of GNPNAT1 in the same anatomical sites of the lung tissue in patients of different LUAD stages are shown. (D) Expression levels of GNPNAT1 in different T stages are shown. (E) Expression levels of GNPNAT1 in different N stages are shown. (F) Survival rate fitting curve of the patients based on the expression of GNPNAT1. The cut-off value for the expression of GNPNAT1 was set to 10. GNPNAT1, glucosamine-phosphate N-acetyltransferase 1; LUAD, lung adenocarcinoma; T, tumor; N, node; M, metastasis.

samples; Wilcoxon test, P=2.4x10^{-29}). The significant differential expression of GNPNAT1 was also observed between tumor and paracarcinoma tissues (Fig. 1B). The expression levels of GNPNAT1 were also associated with clinical stages (Fig. 1C), that is, higher GNPNAT1 expression was associated with advanced clinical stage (stage I vs. stage II vs. stage III vs. stage IV; Kruskal Wallis test, P=5.3x10^{-5}). The slight decrease in GNPNAT1 expression in stage IV compared with stage III could be due to the sole effective radiation therapy in this advanced stage (Fig. 1C). The significant differential expression of GNPNAT1 could be also observed under diverse classification standards. As the tumor proliferated from lung (T1), principle bronchus (T2), chest walls (T3) to heart and great vessels (T4), the expression of GNPNAT1 increased gradually (Fig. 1D). Under a smaller proliferation region, from no proliferation (N0), ipsilateral trachea (N1) to ipsilateral mediastinum (N2), the expression of GNPNAT1 increased gradually, except a slightly decrease at N3 stage (proliferated to contralateral mediastinum) (Fig. 1F). The aforementioned results supported the reliability of GNPNAT1 expression on patient demographics and histories, diagnostic criteria and staging, pathology and even initial treatment. In addition, the results obtained from the western blotting of 35 patients revealed that the protein expression levels of GNPNAT1 in tumor tissues is higher compared with paracarcinoma tissues (Fig. 2). This experimental result was consistent with those observed at the transcriptional level from bioinformatics analysis that both the protein and mRNA expression of GNPNAT1 were higher in the tumor tissues compared with paracarcinoma tissues.

Logistic regression analysis revealed that the expression of GNPNAT1 was associated with the clinical stage of LUAD [odds ratio (OR)=2.92; 95% confidence interval (CI), 1.76-4.96, P-value=4.88x10^{-5}; stage III vs. stage I; Table II]. In addition, the GNPNAT1 high-expression phenotype in tumors was notably associated with vital status (dead vs. alive, OR=1.89), tumor status (tumor status vs. tumor-free status, OR=1.85), N classification (yes vs. no, OR=1.75) and clinical stage (stage II vs.

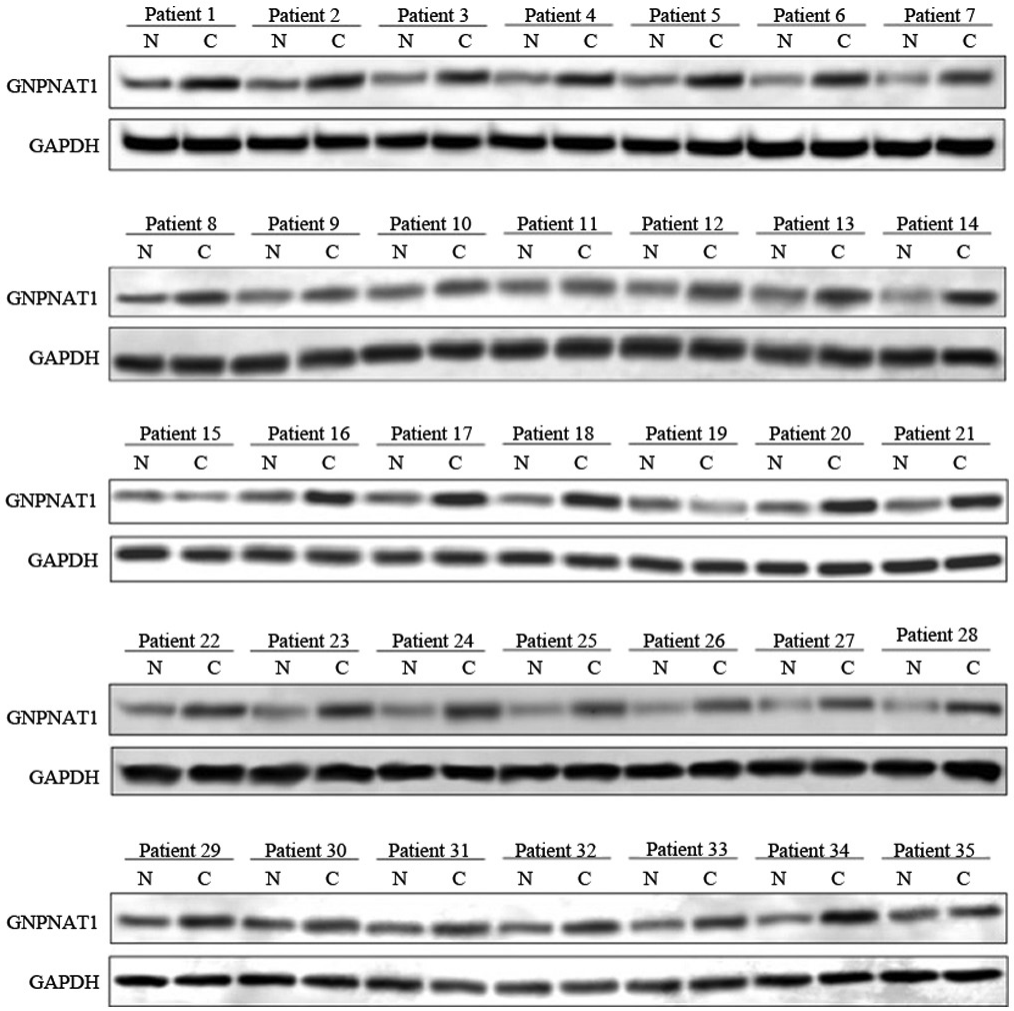

Figure 2. Protein expression levels of GNPNAT1 were determined in tumor and para-carcinoma tissues isolated from 35 patients with LUAD using western blotting. N, para-carcinoma normal tissues; C, tumor tissues; GNPNAT1, glucosamine-phosphate N-acetyltransferase 1; LUAD, lung adenocarcinoma.

Table II. Logistic regression analysis for the association between the expression of GNPNAT1 and clinicopathological characteristics of patients with LUAD.

Clinical characteristics	Total (N)	Odds ratio in GNPNAT1 expression	P-value
Stage (II vs. I)	395	1.66 (1.08-2.56)	0.021
Stage (III vs. I)	358	2.92 (1.76-4.96)	$4.88x10^{-5}$
Status (with tumor vs. tumor-free)	389	1.85 (1.22-2.84)	0.004
Age (≥55 vs. <55 years)	503	0.81 (0.50-1.31)	0.391
Radiation therapy (yes vs. no)	435	1.77 (1.01-3.16)	0.050
Vital status (dead vs. alive)	522	1.89 (1.31-2.73)	0.001
Cigarettes history (yes vs. no)	522	1.28 (0.88-1.86)	0.194
M classification (M1 vs. M0)	378	1.10 (0.48-2.50)	0.825
N classification (yes vs. no)	510	1.75 (1.21-2.55)	0.003
New tumor event after initial treatment (yes vs. no)	425	1.50 (1.00-2.26)	0.051
Disease type (adenomas and adenocarcinomas vs. acinar cell neoplasms adenocarcinomas vs. acinar cell neoplasms	508	1.49 (0.63-3.67)	0.370
Disease type (cystic, mucinous and serous neoplasms vs. acinar cell neoplasms)	36	0.754	0.755

N, node; M, metastasis, GNPNAT1, Glucosamine-phosphate N-acetyltransferase 1; LUAD, lung adenocarcinoma.

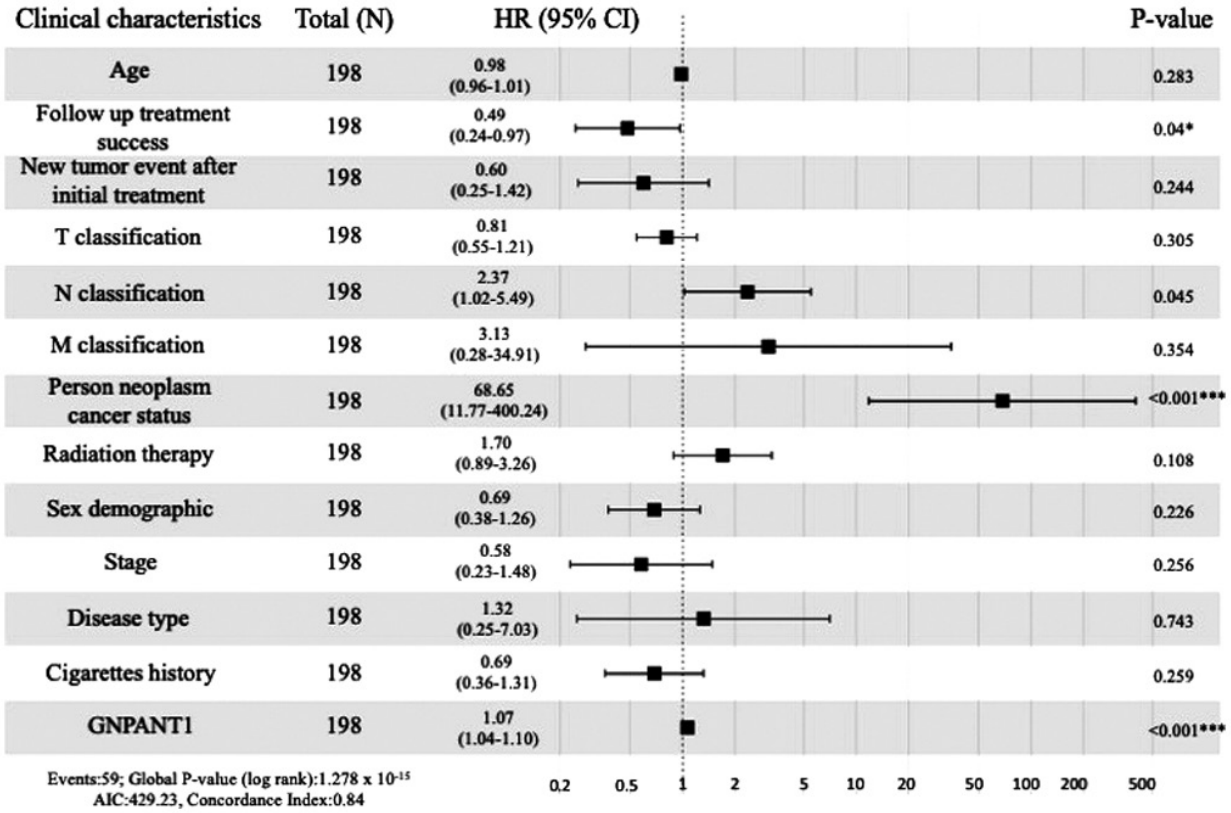

Figure 3. Association between OS and the clinicopathological characteristics of patients with LUAD from TCGA by Cox regression analysis. *0.01<P<0.05, ***P<0.001. OS, overall survival; TCGA, The Cancer Genome Atlas; LUAD, lung adenocarcinoma; T, tumor; N, node; M, metastasis.

stage I, OR=1.66; all P<0.05; Table II). The aforementioned findings indicated that subjects with higher GNPNAT1 expression levels may be more susceptible to LUAD. Additionally, patients with GNPNAT1 high-expression phenotype may be more likely to develop advanced stage LUAD compared with those with lower GNPNAT1 expression levels.

Survival outcomes and multivariate analysis. As shown in Fig. 1F, Kaplan-Meier survival analysis revealed that patients with higher expression of GNPNAT1 exhibited a lower survival rate compared with those with a low expression of GNPNAT1 (Wilcox test, $P=8.9x10^{-5}$). In addition, multivariate analysis demonstrated that the GNPNAT1 high-expression phenotype was significantly associated with cancer status (HR=68.65; 95% confidence interval=11.77-400.24; P<0.001; Fig. 3]. In addition, follow up treatment success was also associated with poor survival in 198 patients who had detailed clinical information (HR=0.49; 95% CI: 0.24-0.97; P-value=0.04; Fig. 3). The above findings further demonstrated that the expression of GNPNAT1 could predict the survival time and regard as a treatment index.

Biological pathways associated with GNPNAT1 expression according to GSEA. GSEA between normal and high GNPNAT1 expression datasets was performed to identify biological pathways differentially enriched in LUAD. A total of 16 significant pathways were found, while only 9 pathways were the most significantly enriched biological pathways, with cut-off values of NOM P-value=0 and FDR q-value ≤0.05 (Table III) in the present study. 'Cell cycle', 'oocyte meiosis', 'pyrimidine mediated metabolism', 'ubiquitin mediated proteolysis', 'one carbon pool by folate', 'mismatch repair', 'progesterone mediated oocyte maturation', 'basal transcription factors', and 'purine metabolism' were the most differentially enriched pathways in the GNPNAT1 high-expression phenotype (Table III). According to the types of these pathways, some of them are prone to somatic mutation, and some of them affect metabolism. This is consistent with the previous observation that somatic cell mutation is high in lung cancer (40).

Discussion

Nothing was previously known about GNPNAT1 in lung carcinomas. Recently, the expression and function of GNPNAT1 in cancer have been extensively reported (31). GNPNAT1 is an essential enzyme involved in the biosynthesis of UDP-GlcNAc and metabolism in eukaryotic cells (28). GNPNAT1 upregulation may affect the occurrence and development of LUAD by disturbing cell metabolism (27). Until now, the expression of GNPNAT1 and its potential prognostic value in LUAD has not been fully investigated. Hence, the present study aimed to evaluate the potential role of GNPNAT1 in LUAD.

In the present study, bioinformatics analysis of the expression data obtained from TCGA demonstrated that increased expression of GNPNAT1 in LUAD was associated with advanced clinical pathologic characteristics (stage, survival status and N classification). To further analyze and reveal the effects of GNPNAT1 expression in LUAD, GSEA was carried out. The analysis revealed that 'cell cycle', 'oocyte meiosis', 'pyrimidine mediated metabolism', 'ubiquitin mediated

Table III. Glucosamine-phosphate N-acetyltransferase 1-related biological pathways according to Gene Set Enrichment Analysis.

Gene set name	NES	NOM P-value	FDR q-value
Cell cycle	2.33	0	$3.24x10^{-4}$
Oocyte meiosis	2.34	0	$4.86x10^{-4}$
Pyrimidine metabolism	2.23	0	$6.55x10^{-4}$
Ubiquitin mediated proteolysis	2.39	0	$9.72x10^{-4}$
One carbon pool by folate	2.06	0	0.009
Mismatch repair	2.02	0	0.013
Progesterone mediated oocyte maturation	1.95	0	0.021
Basal transcription factors	1.95	0	0.022
Purine metabolism	1.96	0	0.023
p53 signaling pathway	1.88	0.002	0.031
DNA replication	1.94	0.002	0.021
Cysteine and methionine metabolism	1.92	0.002	0.022
Aminoacyl tRNA biosynthesis	2.11	0.002	0.005
RNA degradation	2.25	0.002	0.001
Homologous recombination	1.87	0.004	0.031
Nucleotide excision repair	1.86	0.008	0.034

Gene sets with NOM P-value ≤0.05 and FDR q-values ≤0.05 were considered as significant. NES, normalized enrichment score; NOM, nominal; FDR, false discovery rate.

proteolysis', 'one carbon pool by folate', 'mismatch repair', 'progesterone mediated oocyte maturation', 'basal transcription factors' and 'purine metabolism' were enriched in the GNPNAT1 high-expression phenotype. This finding suggested that GNPNAT1 may be regarded as a potential prognostic biomarker and therapeutic target in LUAD. Cell cycle and DNA repair pathways are considered as the 2 most susceptible pathways in LUAD pathogenesis (21). In the cell cycle pathway, the inactivation of several cyclin genes, including CDKN2A, and cyclin-dependent kinases 4 and 6, promotes the escape of cells from the M0 checkpoint, eventually resulting in cellular immortalization, which is a characteristic of cancer cells (41). In the DNA repair pathway, breakdown of the repair system mediates the accumulation of mutations, especially those inactivating tumor suppressor genes and activating oncogenes (42), during the DNA replication process (43). For example, the mutations in AT rich interactive domain 2 results in truncated proteins through out-of-frame indels, nonsense mutations or splice site alterations in hepatocellular carcinoma (44). Somatic intronic mutations of oncogene Met led to an alternatively spliced transcript in lung cancer (45). GNPNAT1 may interfere with these pathways via regulating the activity of cyclin genes through post translational modifications (28). O-linked N-acetylglucosamine (GlcNAc) transferase (OGT) is necessary for the cell cycle since silencing of OGT prevents the synthesis of cyclin D1 (46).

The present study demonstrated that GNPNAT1 may be associated with LUAD carcinogenesis. A previous study demonstrated that silencing of GNPNAT1 attenuated cell proliferation, adhesion, and migration of cancer and fetal human colon cell lines (47). Hence, it was hypothesized that GNPNAT1 upregulation may promote cell migration during carcinogenesis (47). However, the molecular mechanisms underlying LUAD carcinogenesis are still poorly understood. Whether this phenotypic change was directly triggered by GNPNAT1 upregulation remains unknown (48). Hence, cytological evidence is required to further elucidate the biological function of GNPNAT1 in carcinogenesis.

In the future, knockdown of GNPNAT1 in a LUAD animal model could be performed to further evaluate the effects of GNPNAT1 in carcinogenesis. Abraxane®, a FDA approved drug is used to treat advanced breast, lung and pancreatic cancer, and it has been reported to be more effective compared with paclitaxel in the treatment of NSCLC (48). A comparative analysis in A549 lung cancer cells treated in parallel with abraxane and paclitaxel demonstrated that only GNPNAT1 was differentially expressed by 2-fold in A549 cells treated with different drugs (25). This finding indicated that the effects of abraxane may be mediated by GNPNAT1 downregulation, which may cause proliferative delay and cell adhesion defects. Once the role of GNPNAT1 upregulation in LUAD is determined, the screening of more effective and accessible antitumor drugs may be accelerated to benefit all patients suffering from LUAD. Therapeutic intervention based on the effects of GNPNAT1, possibly through mannose analogs, may also have a favorable effect on several diseases, including cancer, which could benefit from suppression of O-GlcNAc signaling and hyaluronan synthesis.

In conclusion, the expression of GNPNAT1 may be a potential and promising diagnostic and prognostic molecular marker of poor survival in patients with LUAD. In addition, the cell cycle and several metabolic pathways, such as pyrimidine metabolism and purine metabolism may be the key pathways regulated by GNPNAT1 in LUAD. However, further validation experiments are needed to verify the biologic effects of GNPNAT1.

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Availability of data and materials

The datasets used and analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

PZ, SG and HH were responsible for conceiving the study, drafting and editing of the manuscript. CZ, ZL and XZ contributed to the acquisition of TCGA data and all the data analysis needed by R packages, and confirmed the authenticity of all the raw data. WW and SX were in charge of patient sample collection, storage and western blotting. KW and TL were responsible for the statistical analysis. YZ was in charge of the study design and manuscript review for important intellectual content. All authors have read and approved the manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of The Second Affiliated Hospital of Nantong University and in compliance with the Declaration of Helsinki. Written patient consent for use of their tissues in research was obtained.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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