



Enhanced expression of queuine tRNA-ribosyltransferase 1 (*QTRT1*) predicts poor prognosis in lung adenocarcinoma

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Background: Lung adenocarcinoma (LUAD) is the most frequently diagnosed type of lung cancer with high percentage of tumor relapse and metastasis. The correlation between queuine tRNA-ribosyltransferase 1 (*QTRT1*) expression and LUAD remains largely unknown. In this study, we aim to investigate the potential role of *QTRT1* expression in the prognosis of LUAD.

Methods: We abstracted data from The Cancer Genome Atlas (TCGA) and four independent Gene Expression Omnibus (GEO) datasets. In total, 1,012 LUAD samples and 112 normal tissue samples were selected. The relationship between *QTRT1* expression, methylation, and clinical features in LUAD were determined, and bioinformatics analyses were also performed.

Results: The expression of *QTRT1* was higher in LUAD patients. A marked downregulation in *QTRT1* methylation in LUAD was also found. Low *QTRT1* expression was associated with longer overall survival across the GEO and TCGA datasets ($P=0.0033$, 0.0022 , respectively). Furthermore, *QTRT1* expression was significantly correlated with ‘axoneme assembly’, ‘androgen response’, and ‘epithelial mesenchymal transition’, as determined by Gene Set Enrichment Analysis (GSEA) and Gene Ontology (GO) term enrichment analysis.

Conclusions: *QTRT1* was highly expressed in LUAD, and enhanced expression of *QTRT1* might therefore serve as a biomarker for poor prognosis in LUAD. The result of bioinformatic analyses might present a new insight for investigating the pathogenesis of LUAD.

Keywords: Lung adenocarcinoma (LUAD); queuine tRNA-ribosyltransferase 1 (*QTRT1*); prognosis; gene expression profile; bioinformatics analysis

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Introduction

Lung cancer is the the leading cause of cancer death worldwide (1). Lung adenocarcinoma (LUAD) is more than any other subtypes of lung cancer (2), and patients with LUAD often die from relapse and metastasis (3-5). Bioinformatics analyses can help to discover novel

biomarkers of LUAD, so as to help improve prognosis and treatment.

Queuine tRNA-ribosyltransferase 1 (*QTRT1*), also known as tRNA-guanine transglycosylase (TGT), is located on chromosome 19p13.2 (6). It contains 10 exons spanning approximately 12 kb. The enzyme queuine tRNA-

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ribosyltransferase (QTRT) is encoded by the gene *QTRT1*. Its molecular function is the exchange of a guanine (G) with queuine (Q) at position 34, resulting in hypermodified transfer RNAs (tRNAs) (7). QTRT1 is a key enzyme involved in the post-transcriptional modification of tRNAs. These tRNAs are central components of protein synthesis and the cell signaling network, and their modulation can specifically impact cancer progression (8). Specific tRNAs are related to human breast cancer, colon adenocarcinoma, and human lung cancer (9-12). At the protein level, western blotting results have shown that QTRT1 expression in the mitochondrion of human LUAD A549 cells was higher than that in normal human bronchial epithelial 16HBE cells. Meanwhile, immunohistochemistry (IHC) results have shown that positive expression of QTRT1 in LUAD was significantly higher compared to normal lung tissues (13). Previous studies have also shown that *QTRT1* may be involved in the carcinogenesis of human colon adenocarcinoma through modulating tRNAs (11). However, the correlation between *QTRT1* and LUAD remains largely unknown, and the specific mechanism of *QTRT1* has not been reported.

In this study, we explored the relationship between *QTRT1* expression, methylation, and clinical features (demographic factors, pathology stage, residual tumor, and survival outcome) in LUAD by using the gene expression data of 1,012 LUAD samples and 112 normal controls. For the first time, QTRT1 was found to be a risk factor for the onset and progression of LUAD. A significant increase in *QTRT1* expression in LUAD patients was observed. Furthermore, a marked down-regulation in *QTRT1* methylation in LUAD was also found. Higher expression of *QTRT1* in LUAD was related to negative survival outcomes. Therefore, *QTRT1* can be used as a novel predictor for survival in patients with LUAD. Furthermore, the biological functions of the identified target genes were interpreted. We present the following article in accordance with the REMARK reporting checklist (available at <http://dx.doi.org/10.21037/atm-20-7424>).

Methods

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Data sources

Data was obtained from The Cancer Genome Atlas (TCGA)

and Gene Expression Omnibus (GEO) sources, namely, microarrays, methylation, and RNA sequencing. A total of 502 LUAD patients with survival outcomes were selected from TCGA, however, 128 patients' clinical information were not available. A total of 374 patients were further selected for analyzing the differences in clinical factors between *QTRT1*^{high} and *QTRT1*^{low} expression groups. The expression level, survival analysis, Gene Ontology (GO) term enrichment, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis, module screening from the protein-protein interaction (PPI) network, and gene set enrichment analysis (GSEA) were also carried out in TCGA. All clinical information (including gender, age, pathological stage, as well as residual tumor) is available in TCGA Data Portal. The project ID is TCGA-LUAD.

Additionally, 510 LUAD samples and 112 control lung tissue samples from 4 GEO databases were further obtained to analyze expression, methylation, and survival status. GSE10072 (n=66) was used for expression analysis, and there were 2 methylation datasets [GSE49996 (n=88) and GSE63384 (n=70)]. In GSE63384, tumor samples and adjacent non-tumor tissues were selected from stage I LUAD. The fourth dataset [GSE72094 (n=398)] was used to assess the relationship between *QTRT1* expression and prognosis.

Microarray analysis

Patients with *QTRT1* expression values above the median for all LUAD cases were classified as high-*QTRT1*, whilst all other cases were considered to be low-*QTRT1*. P value <0.05 in unpaired *t*-test analysis and fold change (FC, log₂) >0.5 or <-0.5 was utilized to determine the differential expression of genes (DEGs).

Functional analysis

GO term enrichment, KEGG pathway analysis, PPI network, and GSEA were constructed to reveal the hub genes of the potential target genes on STRING, a web portal for undermining the integrated function of multiple genes (14,15).

Statistical analysis

R software 3.5.0 was used for performing all statistical analyses. Kolmogorov-Smirnov test was done for evaluating each dataset for normality of distribution to decide whether

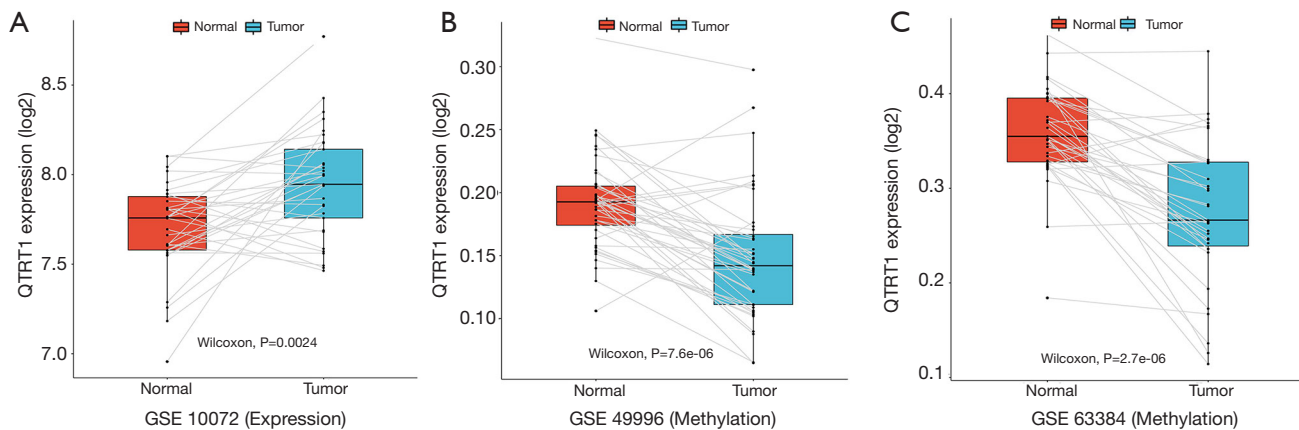


Figure 1 The expression and methylation of queine tRNA-ribosyltransferase 1 (*QTRT1*) in lung adenocarcinoma (LUAD) patients and normal samples. The X-axis represents the different groups: normal group (group 1: red box) and LUAD group (group 2: blue box), while the Y-axis represents *QTRT1* expression and methylation level (log₂). (A) The expression level of *QTRT1* increased in the LUAD group compared to the normal group. (B) The methylation level of *QTRT1* showed a decreasing trend in the LUAD group in the GSE49996 dataset. (C) The same decreasing trend in *QTRT1* methylation level was also found in the LUAD group in the GSE63384 dataset.

a parametric analysis or a non-parametric rank-based analysis should be used. Wilcoxon rank-sum tests and Fisher's exact were used to test hypotheses in continuous and categorical variables, respectively. The limma package was used for performing different gene expression analysis (16). The samples in TCGA were divided into 2 groups (high-*QTRT1*, n=251, low-*QTRT1*, n=251) based on the median expression value of *QTRT1*. GSE72094 was divided into 2 groups (high-*QTRT1*, n=199; low-*QTRT1*, n=199).

For the estimation of the survival status, Kaplan-Meier method and Cox regression multivariate analyses were carried out. And the group comparisons were made by using the log-rank test. Cluster profiler package was used to identify GO enriched terms and KEGG pathways (17). P value <0.05 was considered significant.

Results

Expression and methylation of *QTRT1* in LUAD

We employed 3 GEO datasets to analyze the expression and methylation level of *QTRT1* in LUAD. In the GSE10072 dataset, a significant increase in *QTRT1* expression in LUAD tissues (n=33) was found (P=0.0024) compared to normal control tissues (n=33; *Figure 1A*). An obvious downward trend in *QTRT1* methylation in LUAD tissue was further validated in GSE49996, which included normal tissues (n=44) and LUAD samples (n=44; *Figure 1B*). The same decreasing trend was also found in the GSE63384

dataset in the LUAD group (n=35) compared to the control group (n=35; *Figure 1C*).

Differences in clinical factors in LUAD between the *QTRT1*^{high} and *QTRT1*^{low} groups

The baseline characteristics between the high and low *QTRT1* expression groups were analyzed. Clinical factors included age, gender, stage, residual tumor (R0: without residual tumor, R1: microscopic residual, R2: macroscopic residual tumor, Rx: unknown), tumor size (T) (referring to the TNM stage), lymph node (N) (N0: negative for lymph node metastasis, N1/N2/N3: positive for lymph node metastasis, Nx: unknown lymph node metastasis), and distant metastasis (M) (M0: negative for distant metastasis, M1: positive for distant metastasis, Mx: unknown distant metastasis).

In the 502 LUAD cases selected from TCGA, clinical data from 128 patients were not available. Hence, clinical data from 374 patients were used for analyses. The samples were divided into two groups based on the median values of *QTRT1* expression: *QTRT1*^{low} (n=187) and *QTRT1*^{high} (n=187). Consistent with tumor stage association, patients with higher *QTRT1* expression had less residual tumors (R0 vs. R1/R2/Rx, P=0.029) and less distant metastasis (M0 vs. M1/Mx, P=0.013; *Table 1*). No significant correlation was observed for age (P=0.342), gender (P=0.756), stage (I/II/III/IV, P=0.509), T stage (P=0.071), and N stage (P=0.687; *Table 1*).

Table 1 Clinical characteristics of 374 LUAD patients from TCGA according to *QTRT1* expression levels

Characters	<i>QTRT1</i> ^{low} , n=187	<i>QTRT1</i> ^{high} , n=187	P value
Age, n (%)			0.342
≥60	144 (77.01)	135 (72.19)	
<60	43 (22.99)	52 (27.81)	
Gender, n (%)			0.756
Female	100 (53.48)	96 (51.34)	
Male	87 (46.52)	91 (48.66)	
Stage, n (%)			0.509
I	97 (51.87)	106 (56.68)	
II	43 (22.99)	46 (24.6)	
III	30 (16.04)	26 (13.9)	
IV	14 (7.49)	7 (3.74)	
Residual tumor, n (%)			0.029
R0	172 (91.98)	164 (87.7)	
R1	6 (3.21)	6 (3.21)	
R2	3 (1.60)	0 (0.00)	
RX	6 (3.21)	17 (9.09)	
T, n (%)			0.071
T1	57 (30.48)	68 (36.36)	
T2	107 (57.22)	95 (50.80)	
T3	13 (6.95)	19 (10.16)	
T4	10 (5.35)	3 (1.60)	
Tx	0 (0.00)	2 (1.07)	
N, n (%)			0.687
N0	123 (65.78)	123 (65.78)	
N1	36 (19.25)	38 (20.32)	
N2	26 (13.90)	21 (11.23)	
N3	0 (0.00)	2 (1.07)	
Nx	2 (1.07)	3 (1.60)	
M, n (%)			0.013
M0	139 (74.33)	123 (65.78)	
M1	14 (7.49)	7 (3.74)	
Mx	34 (18.18)	57 (30.48)	

LUAD, lung adenocarcinoma; TCGA, The Cancer Genome Atlas; *QTRT1*, queuine tRNA-ribosyltransferase 1; R0, without residual tumor; R1, microscopic residual tumor; R2, macroscopic residual tumor; Rx, unknown residual tumor; T, tumor size (refer to the TNM stage); N, lymph nodes (N0: negative for lymph node metastasis, N1/N2/N3, positive for lymph node metastasis, Nx, unknown lymph node metastasis); M, distant metastasis (M0, negative for distant metastasis, M1, positive for distant metastasis, Mx, unknown distant metastasis).

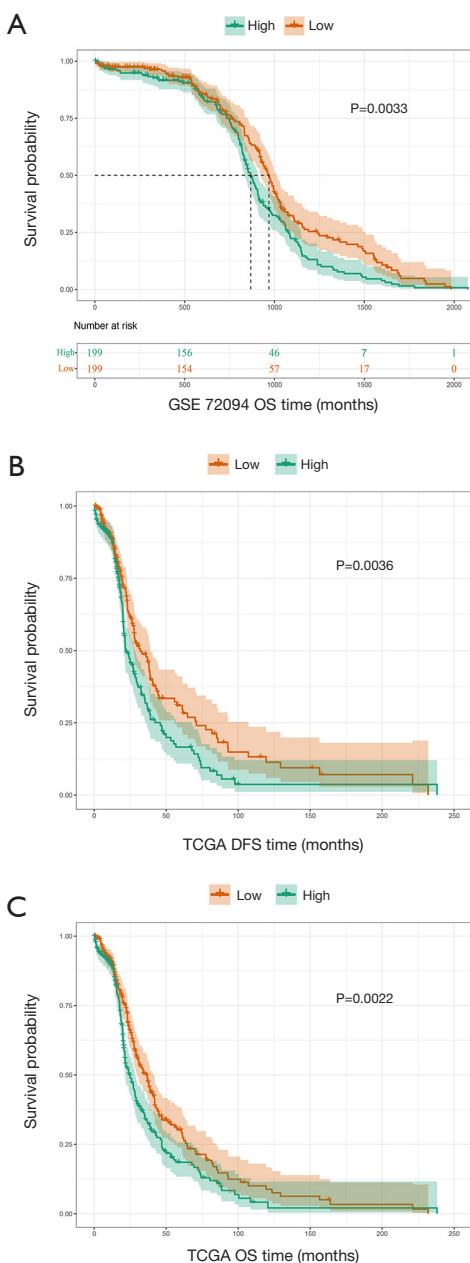


Figure 2 Survival analysis of the queuine tRNA-ribosyltransferase 1 ($QTRT1$)^{high} and $QTRT1$ ^{low} group. The X-axis represents the survival time (months) and the Y-axis represents survival probability. Kaplan-Meier survival curves showed that $QTRT1$ ^{high} predicted worse survival, as per the long-rank test. (A) In the GSE72094 dataset, high expression of $QTRT1$ (n=199) was associated with worse overall survival (OS; P=0.0033). (B) In The Cancer Genome Atlas (TCGA), high expression of $QTRT1$ (n=251) was associated with worse disease-free survival (DFS; P=0.0036). (C) In TCGA, high expression of $QTRT1$ (n=251) was associated with worse OS (P=0.0022).

Association between survival and $QTRT1$ expression in meta-datasets

The GSE72094 dataset (n=398) was divided into 2 groups: high- $QTRT1$ (n=199) and low- $QTRT1$ (n=199). We observed that high expression of $QTRT1$ was associated with worse overall survival (OS; P=0.0033; Figure 2A). Survival analysis was also performed based on TCGA dataset (n=502). Differences were observed in the survival times for the low- $QTRT1$ group (n=251) and the high- $QTRT1$ group (n=251). For disease-free survival (DFS), higher $QTRT1$ expression had a worse outcome (P=0.0036; Figure 2B). Similarly, higher $QTRT1$ expression was associated with significantly shorter OS (P=0.0022; Figure 2C). These results suggest that $QTRT1$ might be an effective gene in the progression of LUAD.

Prognostic value of $QTRT1$ expression in LUAD based on TCGA dataset

Hazard ratios (HRs) were computed for different variables based on TCGA dataset by using the Cox regression model. Univariate analysis results showed that $QTRT1$, $EGFR$, and $KRAS$ were all significantly related to DFS and OS. The HR of the $QTRT1$ ^{high} group was 1.32 (P=0.026) for DFS analysis. The HR of the $QTRT1$ ^{high} group was 1.26 (P=0.031) for OS analysis. $EGFR$ (HR =1.32, P=0.024) and $KRAS$ mutations (HR =1.30, P=0.034) were both risk factors for DFS analysis. Similarly, for OS analysis, $EGFR$ (HR =1.21, P=0.078) and $KRAS$ mutations (HR =1.32, P=0.010) were also negative for survival (Table 2). Other clinical factors such as age, gender, and other genetic alterations (ALK , $LHX5$, $NRAS$, $PIK3CA$, $TP53$) were not significantly different.

Furthermore, in the multivariate analysis for DFS, the HR of $EGFR$ was 1.32 (P=0.034), the HR of $KRAS$ was 1.37 (P=0.016), while the HR of $QTRT1$ expression was 1.37 (P=0.020). These three factors were significantly related to DFS in LUAD patients. The HR of $KRAS$ was 1.38 (P=0.006) and the HR of $QTRT1$ was 1.27 (P=0.044) for OS, indicating that these 2 mutation genes were closely associated with OS. $QTRT1$ expression was a stable risk factor affecting the survival time of LUAD (Table 3). Therefore, these results suggest that $QTRT1$, $EGFR$, and $KRAS$ are involved in the occurrence and development of LUAD, and $QTRT1$ expression can be used as a novel biomarker for the prognosis of LUAD.

Table 2 Univariate analysis for DFS and OS of LUAD based on TCGA dataset

Variables	DFS			OS		
	HR	95% CI	P value	HR	95% CI	P value
QTRT1 (high vs. low)	1.32	1.03–1.68	0.026	1.26	1.02–1.56	0.031
Age (≥ 60 vs. < 60)	1.06	0.82–1.37	0.661	0.97	0.77–1.23	0.808
Gender	0.96	0.75–1.22	0.730	0.93	0.75–1.14	0.475
ALK	0.99	0.78–1.26	0.924	0.95	0.77–1.18	0.648
EGFR	1.32	1.04–1.68	0.024	1.21	0.98–1.49	0.078
KRAS	1.30	1.02–1.66	0.034	1.32	1.07–1.64	0.010
LHX5	0.84	0.66–1.07	0.154	0.86	0.70–1.07	0.172
NRAS	0.88	0.69–1.13	0.315	0.90	0.73–1.12	0.345
PIK3CA	0.99	0.78–1.27	0.944	0.97	0.79–1.20	0.804
TP53	1.11	0.88–1.42	0.380	1.15	0.93–1.42	0.194

DFS, disease-free survival; OS, overall survival; LUAD, lung adenocarcinoma; TCGA, The Cancer Genome Atlas; *QTRT1*, queuine tRNA-ribosyltransferase 1; HR, hazard ratio; CI, confidence interval; *ALK*, anaplastic lymphoma kinase; *EGFR*, epidermal growth factor receptor; *KRAS*, kirsten rat sarcoma viral oncogene; *LHX5*, LIM homeobox 5; *NRAS*, neuroblastoma RAS viral (v-ras) oncogene homolog; *PIK3CA*, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; *TP53*, tumor protein p53.

Table 3 Multivariate analysis for DFS and OS of LUAD based on TCGA dataset

Variables	DFS			OS		
	HR	95% CI	P value	HR	95% CI	P value
QTRT1 (high vs. low)	1.37	1.05–1.79	0.020	1.27	1.01–1.60	0.044
Age (≥ 60 vs. < 60)	1.14	0.87–1.49	0.349	1.03	0.81–1.32	0.801
Gender	1.04	0.81–1.33	0.767	0.97	0.78–1.20	0.750
ALK	0.93	0.73–1.19	0.572	0.92	0.74–1.14	0.439
EGFR	1.32	1.02–1.71	0.034	1.18	0.95–1.48	0.135
KRAS	1.37	1.06–1.78	0.016	1.38	1.10–1.73	0.006
LHX5	0.82	0.64–1.05	0.118	0.85	0.68–1.05	0.129
NRAS	0.90	0.69–1.17	0.422	0.90	0.72–1.13	0.374
PIK3CA	0.99	0.75–1.29	0.923	0.97	0.77–1.23	0.814
TP53	1.03	0.80–1.32	0.821	1.06	0.85–1.32	0.607

DFS, disease-free survival; OS, overall survival; LUAD, lung adenocarcinoma; TCGA, The Cancer Genome Atlas; *QTRT1*, queuine tRNA-ribosyltransferase 1; HR, hazard ratio; CI, confidence interval; *ALK*: anaplastic lymphoma kinase; *EGFR*, epidermal growth factor receptor; *KRAS*, kirsten rat sarcoma viral oncogene; *LHX5*, LIM homeobox 5; *NRAS*, neuroblastoma RAS viral (v-ras) oncogene homolog; *PIK3CA*, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; *TP53*, tumor protein p53.

Differential expression genes between the *QTRT1*^{high} and *QTRT1*^{low} groups

The different expression profiles of *QTRT1*^{high} versus *QTRT1*^{low} were analyzed to find the genes associated with

QTRT1. As many as 101 genes were up-regulated and 157 genes were down-regulated [$P < 0.05$, (FC, log₂) > 0.5 , or < -0.5 , Figure 3A]., The enriched GO terms were analyzed by using DEGs. Among the biological process terms of GO, most of the DEGs were enriched in ‘axoneme assembly’,

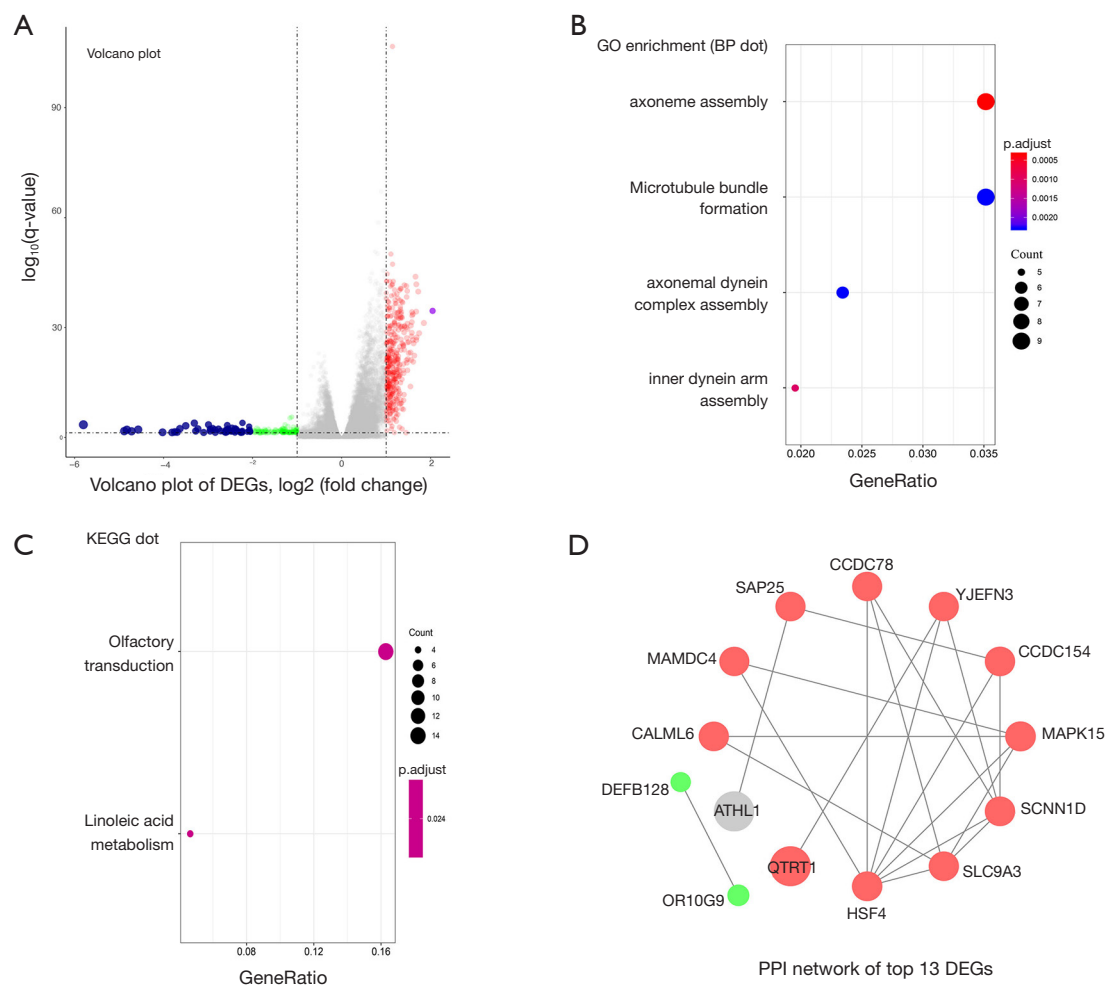


Figure 3 Differential expression genes (DEGs) and their gene enrichment analysis and protein-protein interaction (PPI) analysis. (A) DEGs between the *QTRT1*^{high} group and the *QTRT1*^{low} group were shown in the Volcano plot. The criteria of cut-off for DEGs significance was $P < 0.05$ and absolute value of log₂ fold change > 0.5 . The X-axis displays the log₂ fold change for that gene relative to *QTRT1* expression, while the Y-axis displays the $-\log_{10}$ P value for each gene. (B) Gene Ontology (GO) results for DEGs. The X-axis represents gene ratio and the Y-axis represents different enriched biological process terms. (C) Kyoto Encyclopedia of Genes and Genomes (KEGG) results for DEGs. The X-axis represents gene ratio and the Y-axis represents different enriched pathways. (D) PPI network of top 13 DEGs: 11 up-regulated genes and 2 down-regulated genes. The red circle indicates a positive correlation, and the green circle indicates a negative correlation.

‘microtubule bundle formation’, ‘axonemal dynein complex assembly’, and ‘inner dynein arm assembly’ (Figure 3B). KEGG analysis showed that ‘olfactory transduction’ and ‘linoleic acid metabolism’ were the most enriched pathways (Figure 3C). Top 13 DEGs were used to screen the PPI network in the STRING database. Two down-regulated genes (*DEFB128* and *OR10G9*) and eleven of the up-regulated genes were interactional in the PPI network (Figure 3D).

Hallmark analyses of differential expression genes between the *QTRT1*^{high} and *QTRT1*^{low} groups

While the DEGs can give important clues about the mechanistic aspects of cellular regulation, many genes are regulated in concert. In order to categorize such modules of cellular regulation, bioinformatics approaches for GSEA have been developed (18). We performed GSEA on transcriptomics data downloaded from TCGA. These

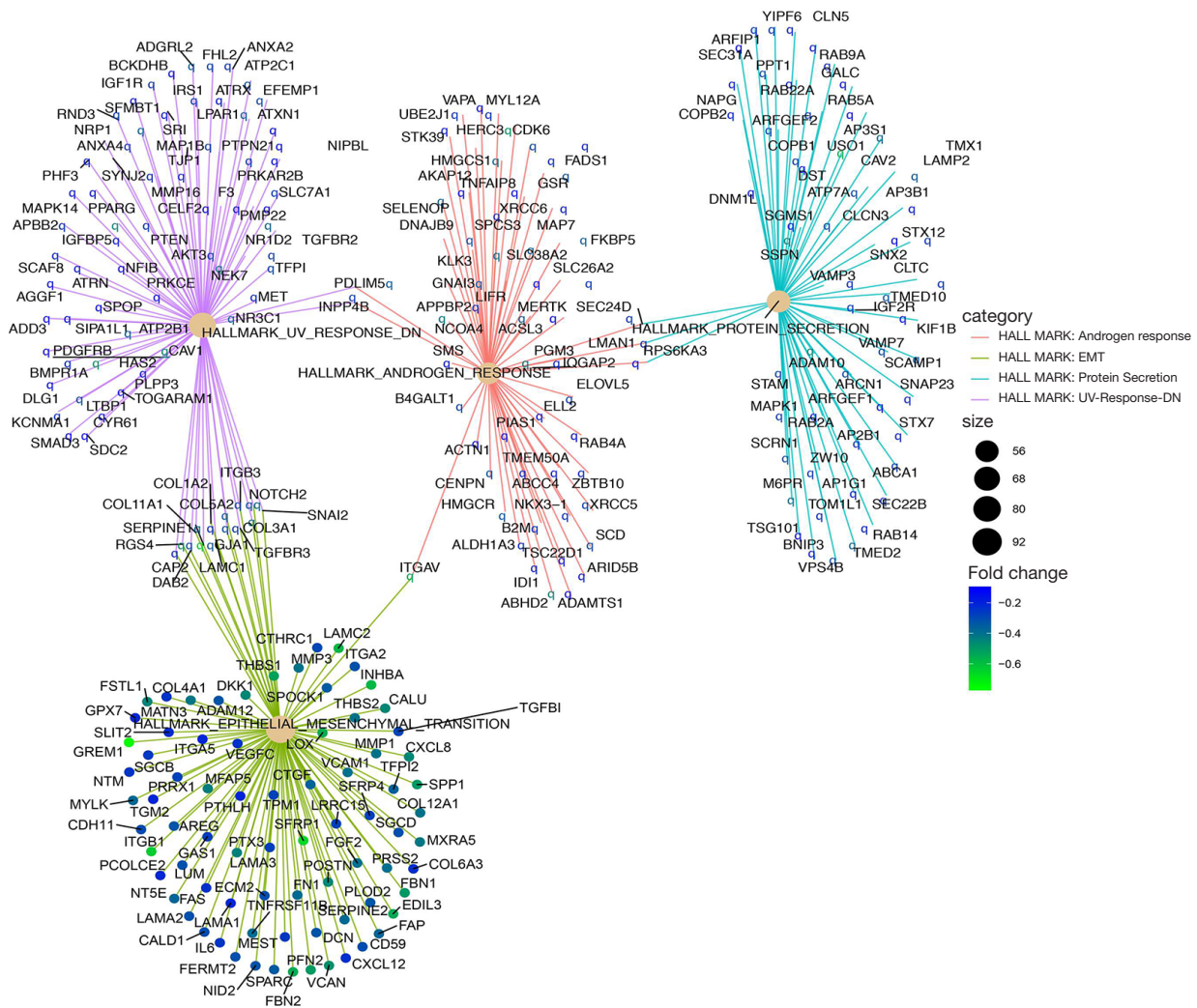


Figure 4 Gene Set Enrichment Analysis (GSEA)-cnetPlot pathway analysis.

genes were involved in the ‘androgen response’, ‘epithelial mesenchymal transition (EMT)’, ‘protein secretion’, and ‘UV-response-DN’ (Figure 4). Significantly enriched gene sets ($P < 0.05$) are shown in Figure 5A,B,C,D.

Discussion

At present, the specific molecular mechanism of *QTRT1* in LUAD remains unknown. A highlight of this study was the use of computational bioinformatics methods to explore the latent mechanism of *QTRT1* in LUAD at the gene level. At the protein level, western blotting results have shown that *QTRT1* expression in the mitochondrion of human LUAD A549 cells was higher than that in the normal

human bronchial epithelial 16HBE cells. Meanwhile, IHC results showed that positive *QTRT1* expression in LUAD was significantly higher than that in normal lung tissues (13). However, there are currently no reports on the correlation between *QTRT1* expression and the clinical features of LUAD at the gene level.

For the first time, *QTRT1* was found to be a risk factor for the onset and progression of LUAD. A significant increase in *QTRT1* expression in LUAD patients was observed compared with normal tissues. An obvious downregulation in *QTRT1* methylation in LUAD was also found. Furthermore, higher expression of *QTRT1* in LUAD was associated with worse prognosis. Therefore, *QTRT1* can be considered a tumor promoter gene in

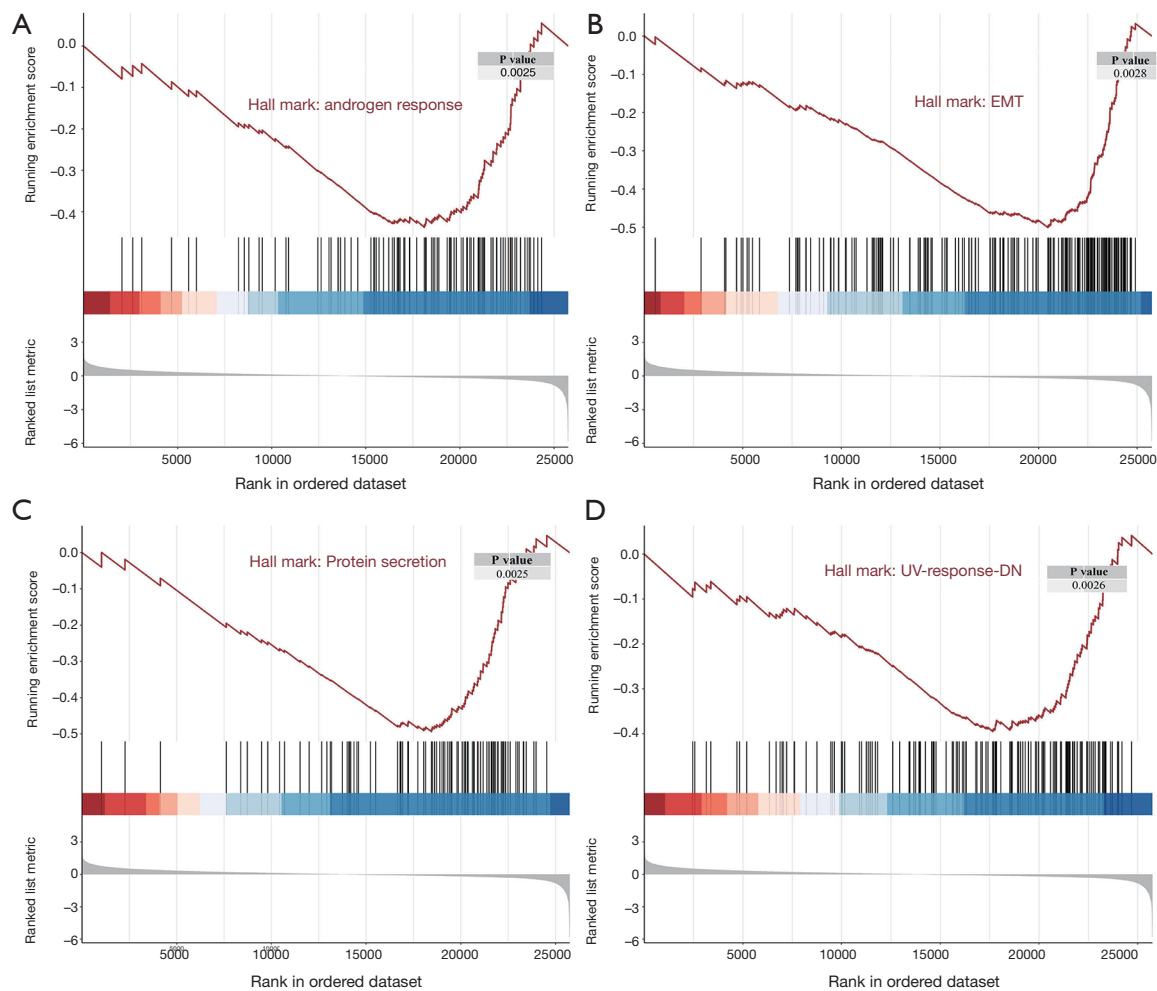


Figure 5 Significant regulated genes by Gene Set Enrichment Analysis. (A) Gene Set Enrichment Analysis (GSEA) plot depicting the enrichment of genes down-regulated in ‘androgen response’ (P=0.0025). (B) GSEA plot depicting the enrichment of genes down-regulated in ‘epithelial mesenchymal transition (EMT)’ (P=0.0028). (C) GSEA plot depicting the enrichment of genes down-regulated in ‘protein secretion’ (P=0.0025). (D) GSEA plot depicting the enrichment of genes down-regulated in ‘UV-response-DN’ (UV-response-DN refers to genes that are down regulated by ultraviolet radiation; P=0.0026).

LUAD, and can be used as a novel predictor of survival in LUAD patients. However, the negative prognostic value of increased expression of QTRT1 of other pathological types of lung cancer have not been found.

QTRT1 is a protein coding gene located on chromosome 19p13.2, and contains 10 exons spanning approximately 12 kb. The enzyme QTRT, also known as TGT, is encoded by the gene *QTRT1*. The active eukaryotic TGT is a heterodimer in solution, comprised of a catalytic QTRT1 subunit and a noncatalytic QTRT2 subunit (6). Previous studies have shown that the molecular function of QTRT is the exchange of a G with Q at position 34, resulting in

hypermodified tRNAs. QTRT is a key enzyme involved in the post-transcriptional modification of tRNAs. It has been reported that *QTRT1* may be involved in the carcinogenesis of human colon adenocarcinoma through modulating tRNAs (11).

These tRNAs have been primarily viewed as static contributors to gene expression. However, they can specifically impact cancer progression, being involved in enhanced invasiveness (8-10,19-21). Goodarzi *et al.* found that specific tRNAs were upregulated in human breast cancer metastasis (22). Q negative tRNA is the original transcript, which contains G at the wobble’s position (the

34th nucleotide from the 5' end). When this G is replaced by Q in a direct base for base exchange, Q negative tRNA is modified to Q positive tRNA (23-25). This modification of tRNAs is one manifestation of the process of glycosylation. Glycosylation is a form of co-translational and post-translational modification (26). It plays an important role in protein folding and stability. Abnormal glycosylation is associated with cell-to-cell adhesion and malignant transformation (27,28). As one of the metabolites, glycosylation is currently considered as a target for cancer treatments (29).

Modification of tRNA was found to be highly correlated with the histopathological grading of human malignant tumors (30,31). The tRNAs of tumors, especially those with poor prognosis and malignant tumors, are often undermodified (32). Chen *et al.* demonstrated that the key role of tRNA modification was to support specific translation during breast cancer progression (7). Shindo-Okada *et al.* found that Q negative tRNA levels in murine erythroleukemic cells decreased markedly when the cells became more differentiated (33). Huang *et al.* reported that Q negative tRNA was found to be highly correlated with the histopathological classification and prognosis of human lung cancer (12). There was also a strong relationship between the malignancy grading of leukemias and lymphomas, and Q positive tRNA (34,35).

Bioinformatics analyses were performed to fully understand the specific molecular mechanism of *QTRT1* in LUAD. According to the GO term enrichment, *QTRT1* might have an important effect on the progression of LUAD by modulating several cellular processes, such as 'axoneme assembly', 'microtubule bundle formation', 'axonemal dynein complex assembly' and 'inner dynein arm assembly'. Optimal cellular function requires the precise regulation of axoneme assembly and membrane biogenesis. Axoneme, microtubules, and dynein are important for maintaining tissue architecture and homeostasis. Disruption of primary cilia structure and function is increasingly recognized in cancers (36). Recent studies have suggested roles for these organelles in oncogenesis and tumor suppression. Zhu *et al.* reported that 'cilium axoneme assembly' was one of key pathways in nasopharyngeal carcinoma (37), and O'Toole *et al.* demonstrated that ciliary disruption contributes to tumorigenesis in endocrine-related cancers (38).

The results of the KEGG analysis also demonstrated the role of *QTRT1* in LUAD. The top 2 enriched pathways were 'olfactory transduction' and 'linoleic acid metabolism'. However, few studies have been conducted on these

pathways in LUAD. Mukhopadhyay *et al.* found that sensory signaling was required to maintain the architecture of the specialized olfactory neuron cilia in *C. elegans*, and decreased sensory signaling resulted in alterations in axoneme length and expansion of the membranous structure (39). A study by Bai *et al.* showed that these pathways were connected mainly to differentially expressed genes, such as low Raps homolog gene family member A (RhoA), and RhoA affects the development of hepatocellular carcinoma (HCC) (40). After integrating the GO and KEGG analyses results above, *QTRT1* might therefore relate to other associated genes (such as *EGFR* and *KRAS*) and play a key role in the occurrence and development of LUAD.

The PPI network results indicated that *QTRT1* was a hub gene in the network. *QTRT1* interacted with many other genes (*YJEFN3*, *SAP25*, *OR10G9*, *HSF4*, *CALML6*, *MAMDC4*, *SLC9A3*, *SCNN1D*, *MAPK15*, and *CCDC154*). Human *YJEFN* domain containing protein family was found to be related to cholesterol processing and steroid hormone metabolism in spermiogenesis and oogenesis (41). IHC of human testes and ovaries showed expression of *hYJEFN3* only in Leydig cells and theca cells, respectively, indicating a role in steroid hormone metabolism (42). Sin3A-associated protein 25 (*SAP25*) was related to ovarian cancer chemoresistance (43). The developing human ovaries express the distinct gene *OR10G9* (olfactory receptor, family 10, subfamily G, member 9) which is related to adrenal and reproductive disorders (44). *HSF4* (heat shock factor 4) is a member of the *HSF* family, and its expression correlates with its role in cell growth and differentiation (45). High *HSF4* expression was an independent prognostic factor of poor OS for colorectal cancer (46). Decreased levels of calmodulin-like 6 (*CALML6*) has been linked to poorer colon cancer patient survival (47), and *MAM* domain containing 4 (*MAMDC4*) was associated with the prognosis of esophageal adenocarcinoma (48). Cancer cells maintain low intracellular pH. *SLC9A3* is involved in pH regulation to eliminate acids generated by active metabolism or to counter adverse environmental conditions. Dorfman *et al.* reported that the *SLC9A3* gene encoded an Na⁺/H⁺ exchanger (49). Kang *et al.* reported that *SLC9A3* was one of the most frequent genetic events in the early stages of non-small cell lung cancer (50), and Bai *et al.* showed that the expression level of *SLC9A3-AS1* in lung cancer was significantly different from healthy controls (51). Meanwhile, *SCNN1* genes encode epithelial sodium channels (ENaCs). ENaCs play a crucial role in maintaining sodium and water balance in the pulmonary

system. Mutations in SCNN1 genes have been shown to affect the structure and function of ENaCs, leading to a series of lung diseases (52). Mitogen-activated protein kinase 15 (MAPK15) is an atypical member of the mitogen-activated protein (MAP) kinase family that has recently emerged as a key modulator of cell transformation. Knockdown of MAPK15 in gastric cancer cells significantly suppressed cell proliferation (53). For lung cancer, Wu *et al.* reported that As₂O₃ can selectively kill lung cancer cells with high MAPK15 expression by activating NF-κB (54). Overexpression of coiled-coil domain-containing 154 (CCDC154) inhibited tumor cell growth by inducing G2/M arrest (55). We therefore hypothesized that *QTRT1* might exert its oncogenic effects in LUAD via some of these genes. As demonstrated in this study, up-regulation of *QTRT1* expression was closely related to the progression of LUAD.

The results of GSEA indicated that *QTRT1* might affect LUAD through ‘androgen response’, ‘epithelial mesenchymal transition (EMT)’, ‘protein secretion’ and ‘UV-response-DN’. The reasons for gender-associated differences in lung cancer mortality are unclear. Although the incidence of lung cancer in men has declined, it had sharply risen in women since 2004 (56,57). Estrogen is a known risk factor for the development of LUAD (58-61). Estrogen can directly stimulate transcription in the nucleus of pulmonary cells and activate growth factor signaling pathways (62). On the other hand, androgen depletion might significantly improve the short-term survival of men with lung cancer (63). Liu *et al.* reported that positive expression of androgen receptors might play a role in the progression and lymph node metastasis of NSCLC, and predict poor prognosis (64). Maßberg *et al.* found that the olfactory receptor OR51E1 [prostate-specific G-protein coupled receptor 2 (PSGR2)] had an impact on androgen receptor-mediated signaling in the growth processes of prostate cancer cells (65). EMT has an important function in the rebuilding of the fibrillar connective tissue and LUAD (66,67). During EMT, epithelial proteins disappear and mesenchymal proteins are expressed. This transition can enhance lung cancer cell invasiveness (68,69). As for ‘protein secretion’, many proteins act as chemokines (a family of small cytokines), and CX3CL1 serves as a prognostic marker for LUAD (70). ‘UV-response-DN’ refers to genes that are down-regulated by ultraviolet (UV) radiation. UV radiation is an omnipresent exposure with confirmed detrimental effects on cancer risk (71). It has been shown to be involved in regulating lung cancer cell apoptosis through the p38

MAPK signaling pathway (72-74).

The current study has limitations. The association between *QTRT1* expression and LUAD should be validated in large prospective trials before it can be translated into clinical practice. The hypothesis that the mechanism involved in the negative regulation of *QTRT1* expression is the methylation of *QTRT1* should be validated. Furthermore, functional studies are required to fully understand the molecular mechanism of *QTRT1* in LUAD progression (cell migration, cell cycle, and animal model experiments), as well as to elucidate its role in pathophysiology. There are no medicine targeting *QTRT1* for the treatment of lung adenocarcinoma right now. *QTRT1* would be a potential target gene therapy in the future medical research. The clinical transformation of the results is a biomarker for early screening of lung adenocarcinoma.

In conclusion, this is the first study to find that *QTRT1* is highly expressed in LUAD. A marked down-regulation in *QTRT1* methylation in LUAD was found, and more importantly, LUAD patients with low *QTRT1* expression consistently had longer survival times. Therefore, our results indicate that high *QTRT1* expression is a biomarker for poor prognosis in LUAD patients, and *QTRT1* may function as a latent tumor biomarker in the prognostic prediction for LUAD. The results of bioinformatics analyses provided new insights into the pathological processes associated with *QTRT1* expression in LUAD. Further studies, including deeper genomic research, are therefore warranted.

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Footnote

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[org/10.21037/atm-20-7424](https://doi.org/10.21037/atm-20-7424)). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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