Original article

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The mRNA of solute carrier family 22, member 17 *(SLC22A17)* plays the potential diagnostic and prognostic role in advantage non-small cell lung cancer patients

L'ARNm de la famille des transporteurs de solutés 22, membre 17 (SLC22A17) joue un rôle potentiel dans le diagnostic le pronostic des patients atteints de cancer du poumon non à petites cellules

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Abstract. *Objective:* This study aims to evaluate the mRNA levels of solute carrier family 22, member 17 *(SLC22A17)* and its potential clinical value as a diagnostic and prognostic biomarker in non-small cell lung cancer. *Methods:* This prospective study measured *SLC22A17* mRNA levels in lung cancer and paracancer tissues using quantitative reverse transcription-polymerase chain reaction (PCR). The levels of *SLC22A17* mRNA in plasma samples from healthy control subjects and patients with lung cancer were also measured. The association between *SLC22A17* mRNA levels in plasma and clinicopathological characteristics was determined. Receiver operating characteristic (ROC) curve analysis was performed to determine the diagnostic value of *SLC22A17* in plasma. Survival curve analysis was performed using the Kaplan-Meier method. *Results: SLC22A17* mRNA levels were significantly higher in lung cancer samples than in the paired paracancerous tissues. Plasma *SLC22A17* mRNA levels were also significantly higher in patients with lung cancer than in healthy controls. The COX analysis indicated that there was a significant correlation between elevated plasma *SLC22A17* mRNA levels and lymph node metastasis, distant metastasis, and TNM stage. Furthermore, the ROC curve analysis demonstrated that plasma *SLC22A17* had high diagnostic value. High plasma *SLC22A17* mRNA levels are associated with a significantly shorter survival time. *Conclusion: SLC22A17* is upregulated in lung cancer and may serve as a novel diagnostic and prognostic biomarker

Key words: *SLC22A17, lung cancer tissues, biomarker, prognosis.*

Résumé. *Objectif :* Cette étude vise à évaluer les niveaux d'ARNm du transporteur de soluté de la famille 22, membre 17 *(SLC22A17)* et sa valeur clinique potentielle en tant que biomarqueur diagnostique et pronostique dans le cancer du poumon non à petites cellules. *Méthodes :* Cette étude prospective a mesuré les niveaux d'ARNm du *SLC22A17* dans des tissus de cancer du poumon et de paracancer à l'aide d'une transcription inverse quantitative et d'une réaction en chaîne de la polymérase (PCR). Les niveaux d'ARNm *SLC22A17* dans les échantillons de plasma de sujets témoins sains et de patients atteints de cancer du poumon ont également été mesurés. L'association entre les niveaux d'ARNm *SLC22A17* dans le

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plasma et les caractéristiques clinico-pathologiques a été déterminée. Une analyse de la courbe ROC *(Receiver Operating Characteristic)* a été réalisée pour déterminer la valeur diagnostique de *SLC22A17* dans le plasma. L'analyse de la courbe de survie a été réalisée à l'aide de la méthode Kaplan-Meier. *Résultats :* Les niveaux d'ARNm de *SLC22A17* étaient significativement plus élevés dans les échantillons de cancer du poumon que dans les tissus paracancéreux appariés. Les niveaux d'ARNm *SLC22A17* dans le plasma étaient également significativement plus élevés chez les patients atteints de cancer du poumon que chez les témoins sains. L'analyse COX a montré qu'il existait une corrélation significative entre les niveaux élevés d'ARNm *SLC22A17* dans le plasma et les métastases ganglionnaires, les métastases à distance et le stade TNM. En outre, l'analyse de la courbe ROC a démontré que le *SLC22A17* plasmatique avait une valeur diagnostique élevée. Des niveaux élevés d'ARNm *SLC22A17* dans le plasma sont associés à une durée de survie significativement plus courte. *Conclusion : SLC22A17* est régulé à la hausse dans le cancer du poumon et peut servir de nouveau biomarqueur diagnostique et pronostique.

Mots-clés : *SLC22A17, tissus de cancer du poumon, biomarqueur, pronostic.*

Introduction

Lung cancer is the most common type of cancer worldwide and the main cause of cancer-related death [1, 2]. Notwithstanding significant advancements in targeted therapy and immunotherapy in the last few years [3], the majority of patients with lung cancer have a poor prognosis due to an increased incidence of metastasis and recurrence [4]. Non-small cell lung cancer has a poor prognosis, and several patients are diagnosed with progressive disease or distant metastases at the initial diagnosis [5]. Therefore, it is critical to develop novel biomarkers and therapeutic targets to improve lung cancer diagnosis and prognosis.

Given the Fe (III) is very important for multiple biochemical processes, iron metabolism-related genes perform various metabolic tasks, including cell proliferation and immunological modulation [6]. Solute carrier family 22, member 17 (*SLC22A17*, NGALR) is a well-known specific cell surface receptor of LCN2 that contributes to remodel the tumor microenvironment (TME) and affect the tumor progression [7]. *SLC22A17* has lately been implicated in the development of numerous types of cancer, acting as an oncogene in gliomas, clear cell renal cell carcinoma, pancreatic ductal adenocarcinoma, esophageal squamous cell carcinoma and gastric cancer [8-11]. Additionally, this protein is effective in the diagnosis and prediction of prognosis for these tumours [12, 13]. *SLC22A17*/*LCN2* appears to play a key role in tumour progression when combined with metalloproteinase [7], generating gelatinolytic activity and promoting epithelial-to-mesenchymal transition (EMT) [14-16]. *SLC22A17* expression seems to increase in ovarian cancer, where the protein appears to play a role in tumour differentiation [17]. However, the exact function of *SLC22A17* in lung cancer remains unknown.

The current study aimed to evaluate the expression profile of the *SLC22A17* gene and its potential clinical value as a diagnostic and prognostic biomarker for lung cancer.

Materials and methods

Tissues and plasma specimens

This retrospective study was used the collected paired lung cancer and paracancer tissues from the Department of Respiratory Medicine, Fourth Hospital of Hebei Medical University, Shijiazhuang, Hebei Province, China, between December 2013 and June 2016. Plasma samples were obtained from treatment-naive lung cancer patients and healthy control subjects attending a clinic for routine examination at the Department of Respiratory Medicine, The Fourth Hospital of Hebei Medical University, between May 2014 and July 2017. All surgical tissue and plasma samples were collected prior to therapy at the time of diagnosis.

The Ethics Committee of the Fourth Hospital of Hebei Medical University authorized the study (no. 2022KS019). All the study participants provided written informed consent. This retrospective study conformed to the STROBE guidelines [18].

Quantitative RT-PCR

Total RNA was extracted using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Total RNA (2 mg) was reverse transcribed using a moloney murine leukaemia virus reverse transcriptase kit (Promega, Madison, WI, USA). *SLC22A17* mRNA levels were determined by quantitative reverse transcription-polymerase chain reaction (qRT-PCR) using the SYBR Green Master PCR Mix (Applied Biosystems, Foster City, CA, USA) and a TP800 Thermal Cycler DiceTM Real Time System (TaKaRa, Dalian, China). Primers were provided by Generay Biotech (Shanghai, China). Glyceraldehyde 3-phosphate dehydrogenase *(Gapdh)* was used as the internal control. The following primer sequences were used: 5'-GCCCAGGACTCAACTCAGAA-3' (sense) and 5'-GACCAGGATGGAGGTGACAT-3' (anti-sense) for *SLC22A17*; 5'-GGACCTGACC-TGCCGTCTAG-3' (sense) and 5'- GTAGCCCAG-GATGCCCTTGA-3' (antisense) for *Gapdh*. The cycling program involved preliminary denaturation at 94°C for 1 min; followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30 s, and elongation at 72°C for 45 s; followed by a final elongation step at 72°C for 5 min. The levels of *SLC22A17* mRNA were determined using the relative level of $2^{-\Delta\Delta Ct}$ method. The Ct value used for these calculations was the mean of triplicate measurements for each reaction.

The Cancer Genome Atlas (TCGA) data set

TCGA analysis was performed to explore the levels of *SLC22A17* mRNA in tumour and paracancer tissues from patients with lung cancer. 200 individual gene expression profiles of NSCLC patients with paired paracancer from The Cancer Genome Atlas (TCGA) were also downloaded.

Human Protein Atlas (HPA) database analysis

HPA database analysis was performed to detect *SLC22A17* protein levels in the tumour and normal tissues.

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 20.0 (IBM Corp., Armonk, NY, USA). Data was presented as mean ± SD. Student's t-test or one-way analysis of variance was used to compare the different groups. Categorical data was presented as n of patients and compared by the χ^2-test. Receiver operating characteristic (ROC) curve analysis was used to evaluate the diagnostic value of *SLC22A17* using GraphPad Prism7 (Graphpad Software Inc., San Diego, CA, USA). The survival curves and features were calculated using the Kaplan-Meier method and log-rank test. Univariate and multivariate COX analysis were also performed to investigate the effect of sex, age, TNM stage, clinical stage and the *SLC22A17* expression$^{high/low}$ on the overall survival rate. The statistical significance level was set at $p < 0.05$.

Results

SLC22A17 *was overexpressed in lung cancer tissues and plasma*

This study collected 54 paired lung cancer samples and the paracancer tissues. Quantitative RT-PCR was used to determine the levels of *SLC22A17* mRNA in paired lung cancer tissues and para-cancer tissues. The result indicated that the *SLC22A17* mRNA levels were significantly higher in lung cancer samples than in the paired paracancer tissues ($P < 0.001$) (*figure 1A*). In order to identify the SLC22A17 expression profile in a larger cohort, TCGA dataset analysis was employed and the result indicated that *SLC22A17* levels were also significantly higher in lung cancer samples than in paracancer tissues (*figure 1B*; Supplementary Materials, *figure 1*). HPA database analysis demonstrated that *SLC22A17* protein levels were higher in tumour tissues than in normal tissues (see Supplementary Materials, *figure 2*).

Plasma samples were obtained from 80 treatment-naive lung cancer patients and 46 healthy controls. Plasma *SLC22A17* mRNA levels in patients with lung cancer and healthy control subjects were analysed using qRT-PCR. These analyses demonstrated that lung cancer patients had significantly higher plasma *SLC22A17* mRNA levels than healthy control subjects ($P < 0.001$) (*figure 1C*).

Relationships between SLC22A17 *levels in tissue or plasma and clinicopathological factors in patients with lung cancer*

The association between *SLC22A17* mRNA levels and clinicopathological features was investigated. After the COX analysis, the *SLC22A17* mRNA levels in lung cancer tissues were significantly correlated with patients with N2-N3 lymph node metastasis ($P = 0.046$) and stage III-IV lung cancer ($P = 0.029$) (*table 1*). No other significant associations were found between *SLC22A17* mRNA levels in lung cancer tissues and other clinical variables, including sex, age, tumour differentiation, tumour size, or distant metastasis. Plasma *SLC22A17*

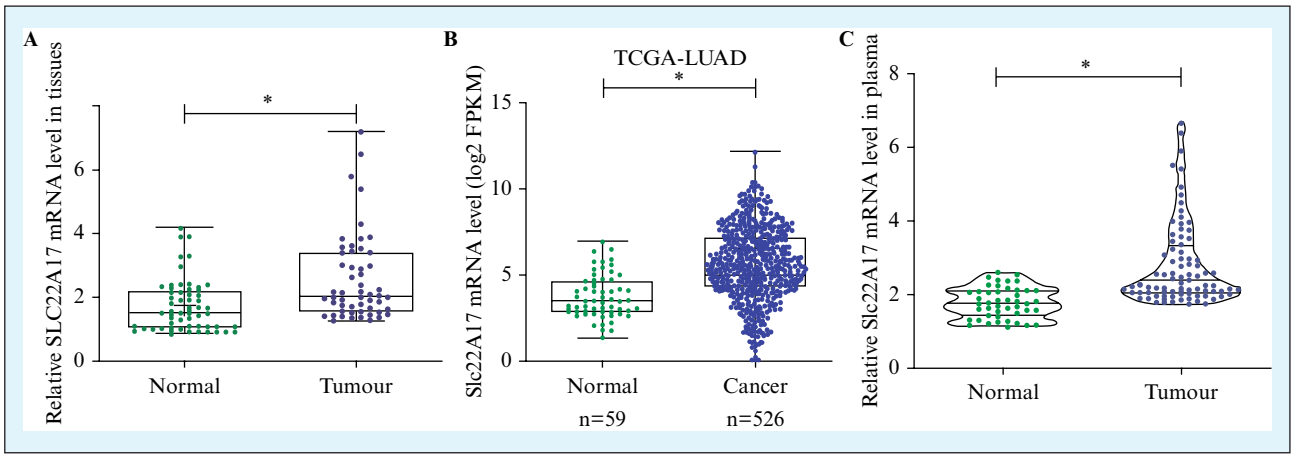

Figure 1. Relative *SLC22A17* expression in tumor tissues and plasma of lung cancer patients. (A) *SLC22A17* expression in lung cancer tissues in comparison to paired paracancer tissues (n = 54). (B) The expression of *SLC22A17* was compared between lung cancer and normal tissues using the TCGA lung cancer (LUAD) database. (C) Plasma *SLC22A17* levels were significantly higher in lung cancer patients than in healthy controls. ***P < 0.001.

mRNA levels were significantly higher in patients with N2-N3 lymph node metastasis (P = 0.030), the distant metastasis (P = 0.014), the stage III-IV lung cancer (P = 0.044) (*table 2*). No significant associations were found between plasma *SLC22A17* mRNA levels and other clinical variables, including sex, age, tumour differentiation, or tumour size. Therefore, there is highly consistance of the expression and clinical factors association between the *SLC22A17* mRNA levels in tissues and plasma.

Plasma SLC22A17 *served as a diagnostic biomarker for lung cancer*

Given the more convenient clinical accessibility of *SLC22A17* mRNA levels in plasma, the plasma level of *SLC22A17* was used to evaluate its diagnostic value. A ROC curve analysis was used to determine the diagnostic utility of plasma *SLC22A17* mRNA levels. The accordingly area under the curve (AUC) was 0.842 (95% confidence interval (CI): 0.773-0.912) (*figure 2*).

Prognostic significance of plasma SLC22A17 *for lung cancer*

The NSCLC patients were stratified into low and high groups according to plasma *SLC22A17* mRNA levels based on the median plasma *SLC22A17* mRNA level (the relative expression value is 2.384). The overall survival time of patients with high plasma *SLC22A17* mRNA level was significantly shorter than that of patients with a low plasma mRNA level (P = 0.0004), as determined by Kaplan-Meier curves and the log-rank test (*figure 3A*). Furthermore, COX analysis was employed to verify the effect of $SLC22A17^{high}$ expression on the clinical survival rate in different TNM cohort, the results indicated that III-IV stage as well as $SLC22A17^{high}$ expression contributed to shorter overall survival time in the NSCLC subjects (HR = 2.137, 95%CI ~ 1.133-3.487) (*figure 3B-C*).

Discussion

LCN2 and its receptor SLC22A17 always co-express in a large number of proliferating cells, implying that they may be intimately involved in proliferation processes [19]. However, the previous literature indiscriminately report the potential role of SLC22A17 and LCN2 in proliferation. While the SLC22A17 protein also seems to be effective in the diagnosis and prognosis of various malignancies [12, 13]. Therefore *SLC22A17* is a potential biomarker for tumour diagnosis and prognosis. There were fewer reports about its diagnosis role in cancers than *Lcn2*. The current study demonstrated that *SLC22A17* mRNA levels were significantly increased in lung cancer tissues compared with paracancer tissues and in the plasma of patients compared with healthy control subjects. It is worth noting that the *SLC22A17* overexpression, TNM stage and distant metastases are independent prognostic variables indicating shorter survival time in patients with lung cancer [20-22]. Our result indicated that *SLC22A17* is involved in tumour metastasis and may be a useful prognostic marker for lung cancer patients.

Table 1. The associations between *SLC22A17* expression in tissues and clinicopathological variables in lung cancer patients.

Characteristics	Group	Total (n = 54)	*SLC22A17* expression[a]		*P* value[b]
			High (n = 27)	Low (n = 27)	
Gender	Male	17	11	6	0.143
	Female	37	16	21	
Age (years)	< 60	29	15	14	0.785
	≥ 60	15	12	13	
Differentiation	Middle-high	16	7	9	0.551
	Poor	38	20	18	
Tumor size (cm)	≤3	18	8	10	0.564
	>3	36	19	17	
Lymph node metastasis	N0-N1	19	6	13	0.046*
	N2-N3	35	21	14	
Distant metastasis	Yes	49	24	25	0.806
	No	25	13	12	
TNM stage	I, II	28	10	18	0.029*
	III, IV	26	17	9	

Note: *Significant difference
[a] Patients were stratified into a low and high group according to the tissue *SLC22A17* mRNA levels based on the median tissue *SLC22A17* mRNA level of 2.03.
[b] χ^2-test; NS, no significant association (P ≤ 0.05). TNM, tumour, node, metastasis.

Table 2. The correlations between *SLC22A17* levels in plasma and clinicopathological characteristics of lung cancer patients.

Characteristics	Group	Total (n = 80)	*SLC22A17* expression		*P* value
			High (n = 40)	Low (n = 40)	
Gender	Male	27	15	12	0.478
	Female	53	25	28	
Age (years)	< 60	23	11	12	0.805
	≥ 60	57	29	28	
Differentiation	Middle-high	18	6	12	0.108
	Poor	62	34	28	
Tumor size (cm)	≤ 3	18	6	12	0.108
	> 3	62	34	28	
Lymph node metastasis	N0-N1	55	23	32	0.030*
	N2-N3	25	17	8	
Distant metastasis	Yes	63	27	36	0.014*
	No	17	13	4	
TNM stage	I, II	37	14	23	0.044*
	III, IV	43	26	17	

Note: *Significant difference
[a] Patients were stratified into a low and high group according to the plasma *SLC22A17* mRNA levels based on the median plasma *SLC22A17* mRNA level of 2.384.
[b] χ^2-test; NS, no significant association (P ≤ 0.05). TNM, tumour, node, metastasis.

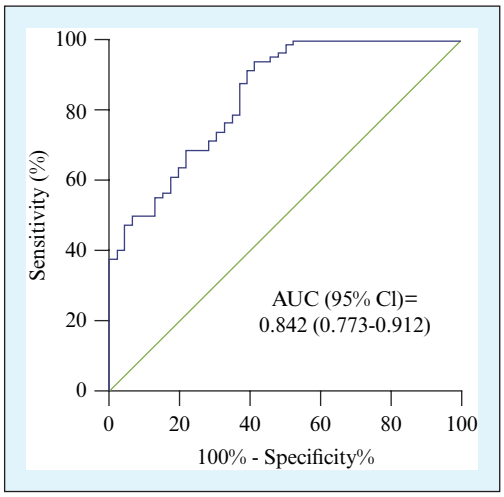

Figure 2. ROC curve of plasma *SLC22A17*. The AUC value of *SLC22A17* is 0.842 (95% CI: 0.773-0.912) to discriminate patients with lung cancer from healthy controls.

Emerging evidence has established that plasma *SLC22A17* can be used as a highly sensitive molecular biomarker for cancer diagnosis. For example, *SLC22A17* is associated with poor outcomes in HCC cancer [23]. Wang *et al*. established that plasma *SLC22A17* and *SUPV3L1* could be employed as hub genes for the prognostic of gastric cancer [24]. The *SLC22A17* and Lcn2 were associated with series of cellular function. Both *SLC22A17* and Lcn2 were regarded as key modulators to transport small lipophilic ligands, regulate tissue development and differentiation [25]. In previous study, stronger Lcn2 staining were observed in well- or moderately-differentiated samples than poorly-differentiated samples [26]. They can also contribute to metastasis by reducing cell-cell adhesion and reshaping extracellular matrix [27, 28]. The ROC curve analysis were also performed to determine the diagnostic value of plasma *SLC22A17* mRNA levels in this study. As illustrated in *figure 2*, the area under the curve for plasma *SLC22A17* mRNA is 0.842. This finding indicated that plasma *SLC22A17* mRNA levels may be a potential biomarker indicating tumor risk without lung lesions puncture examination.

Additionally, *SLC22A17* has been documented to be a possible predictive factor for survival in various endometrial cancers [17, 29, 30]. In the current study, lung cancer patients with high plasma *SLC22A17* mRNA levels had a significantly shorter survival time than those with low plasma *SLC22A17* mRNA levels (P = 0.0004). Therefore the plasma *SLC22A17* mRNA levels were revealed to be negatively associated with overall survival rate (OS) in patients with lung cancer based on Kaplan-Meier curves and the log-rank test.

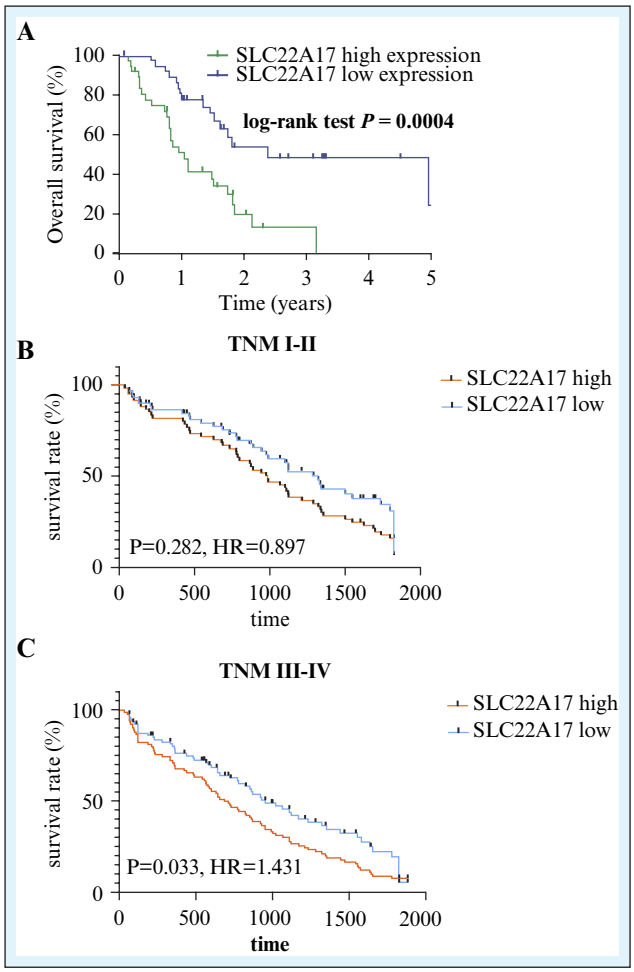

Figure 3. (A) Kaplan-Meier survival curves for patients with lung cancer. Patients with lung cancer with a high level of plasma *SLC22A17* expression had a shorter overall survival (OS) than those with a low level of *SLC22A17*. The OS survival curve for TNM stage I-II (B) and III-IV (C). The log-rank test was performed to evaluate the HR score and 95% CI. The Mantel-Cox test was performed to evaluate statistical significance.

There are several limitations in this study. First, the number of subjects enrolled in this study was relative small. Further research in a larger patient population is required to verify these preliminary findings. Second, the stepwise functional mechanisms of the *SLC22A17* gene in lung cancer require further experimental verification.

Conclusion

In conclusion, *SLC22A17* mRNA level has been detected to be significantly increased in lung cancer tissues and in the plasma of patients in our study.

High plasma *SLC22A17* mRNA level was also shown to be associated with N2-N3 lymph node metastasis, distant metastasis, and TNM stage in the current investigation. The current findings suggest that plasma *SLC22A17* mRNA level merits favourable sensitivity, specificity, and accuracy, and it can be used as a noninvasive diagnostic biomarker for lung cancer. The high *SLC22A17* mRNA levels in plasma might also be useful biomarkers for lung cancer indicating a shorter overall survival time.

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Author contributions: Wen-Wen Qin contributed to the conception and design; Cui-Min Ding contributed to the analysis and interpretation of the data in the original version, and contributed to the design of the work. Fei Peng contributed to the acquisition, analysis, and interpretation of the data and the drafting of the paper; Ping Wang contributed to the revision, including the design of the work, the acquisition, analysis, and interpretation of data in revision; Wen-Wen Qin contributed to the conception and design of the work, the interpretation of data, modification of the manuscript, and the final approval of the version to be published. All authors agree to be accountable for all aspects of the work.

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