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Effect of lncRNA MALAT1 expression on survival status of elderly patients with severe pneumonia

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Abstract. – OBJECTIVE: The aim of this study was to explore the relationship between serum MALAT1 level and clinical features of elderly patients with severe pneumonia and its impact on patients' survival.

PATIENTS AND METHODS: A total of 150 elderly patients with severe pneumonia were enrolled in this study. According to patients' prognosis, enrolled subjects were divided into two groups, including death group (n=63) and survival group (n=87). The clinical data and indicators of subjects were collected, and χ^2 and *t*-tests were used for statistical analysis. MALAT1 expression in the serum of all subjects was examined through the qPCR assay. Meanwhile, the predictive value of MALAT1 for patient death was assessed by the receiver operating characteristic curve (ROC).

RESULTS: PT, APTT, DD, APACHE II scores, and MODS scores in death group were remarkably higher, while HB, HCT, TT, and PaO_2/FiO_2 were conversely lower than those in survival group ($p<0.05$). QRT-PCR results revealed significantly increased MALAT1 expression in death group when compared with survival group, especially in those patients with a history of smoking and COPD ($p<0.05$). In addition, ROC analysis confirmed the predictive value of MALAT1 for the prognosis of elderly patients with severe pneumonia.

CONCLUSIONS: MALAT1 is highly expressed in the serum of elderly patients with severe pneumonia. Furthermore, it may serve as a marker for the prediction of survival of these patients.

Key Words:
Senile severe pneumonia, MALAT1, Prognosis, Biomarkers.

Introduction

Chronic obstructive pulmonary disease (COPD) is a common and frequently-occurring respiratory disease. The prevalence of COPD in people over 40 years old is as high as 10% and is still on the rise, making it a global public health problem. Severe pneumonia can result in multiple organ dysfunction syndrome (MODS). Currently, acute physiology and chronic health condition scoring system (APACHE), MODS scoring, pneumonia severity (PSI) scoring, and other systems are commonly used in clinical evaluation[1]. However, the calculation of these scoring systems is relatively cumbersome, seriously limiting their application[2]. Therefore, it is of great significance to search for effective and simple biomarkers for the clinical diagnosis and treatment of severe pneumonia.

Long non-coding RNAs (lncRNAs) are a kind of non-protein RNA transcripts greater than 200 nt in length; they are located in antisense transcripts that extend or overlap between genes encoding a protein-encoding gene. LncRNAs are closely related to chromosome modification, transcriptional activation or mRNA degradation. Meanwhile, they are not only involved in normal physiological cell activities, but also in disease progression and prognosis[3-5]. With the rapid development of RNA genomics, a great number of lncRNAs have been found engaged in the development of COPD, such as LISPR1 and ANRIL[6,7]. LncRNA TUG1 promotes airway remodeling *via* inhibiting the miR-145-5p/DUSP6 axis in COPD induced by cigarette smoke[8]. However, the correlation between elderly severe pneumonia and lncRNA has not been fully elucidated.

Metastatic related lung adenocarcinoma transcript 1 (MALAT1), also known as NEAT 2, HCN, LINC00047, NCRN00047, and PRO2853, is one of the most abundant and highly conserved lncRNAs located in human chromosome 11q13. The length of MALAT1 RNA is 87 kb, which

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comes from a single exon and is mainly located in cell nucleus[9]. MALAT1 may have complex and extensive functions in the progression of malignancies[10,11]. MALAT1 was initially identified as a transcript associated with the metastasis and survival of non-small cell lung cancer (NSCLC) patients, suggesting its crucial biological function[9,12]. Therefore, in this study, we explored the influence of serum MALAT1 level on patients' condition and survival. Our findings might help to provide a new idea for clinical diagnosis, treatment, and research of severe pneumonia in the elderly.

Patients and Methods

Normal Information

This investigation was approved by the Ethics Committee of Shanghai Pulmonary Hospital. Signed written informed consents were obtained from all participants before the study. 150 elderly patients with severe pneumonia admitted to our hospital from May 2016 to December 2018 were enrolled in this study. Inclusion criteria: (1) All patients met the diagnostic criteria for severe pneumonia jointly developed by the American Society of Infectious Diseases (IDSA)/American Thoracic Society (ATS) in 2007. The main indicators included: 1. patients with septic shock, needed to be given blood vessels contraction drug; 2. mechanical ventilation was required. Secondary indicators: 1. oxygenation index [arterial oxygen partial pressure (PaO_2)/inhaled oxygen concentration (FiO_2)] $\leq$ 250; 2. respiratory rate (RR) $\geq$ 30 times/min; 3. inflammation cumulative multiple lung lobe; 4. Obstacle or confusion; 5. blood urea nitrogen (BUN) level $\geq$ 200 mg / L; 6. blood white blood cell (WBC) count $< 4 \times 10^9$/L; 7. platelet (PLT) count $<10 \times 10^9$/L; 8. body temperature (T) <36°C; 9. hypotension occurred, a strong body fluid resuscitation was required. Patients with severe pneumonia could be diagnosed if they met one of the above-mentioned primary criteria or three secondary criteria[13]. (2) Age $\geq$ 60 years old. (3) Clinical data was complete and reliable. Patients suffered from hepatitis, liver cirrhosis, liver cancer, and other liver diseases or those combined with carbamate or organophosphorus pesticide poisoning were excluded. 63 patients died of severe pneumonia or complications during hospitalization were classified as death group. Meanwhile, the remaining 87 patients cured at discharge were classified as survival group.

RNA Extraction

First, the separated serum was melted on ice. 250 μL serum samples were taken and added with 750 μL TRIzol reagent (Invitrogen, Carlsbad, CA, USA). After adding 200 μL chloroform, the samples were mixed with oscillator and let stand for 10 min. After centrifugation at room temperature at 12 000 r/min for 10 min, the supernatant was transferred to another Eppendorf (EP) tube without RNAse. Next, isopropanol of the same volume as the supernatant was separately added and mixed well with an oscillator. Lastly, total RNA concentration and purity were determined by spectrophotometer.

Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

Total RNA was extracted, and reverse transcription reaction was carried out according to the instructions of AMV reversal kit. 2 μg of total RNA was added to a 20 μL system for complementary deoxyribose nucleic acid (cDNA) synthesis. QRT-PCR was carried out using 2xSYBR Green PCR Master Mix, with an appropriate amount of cDNA as template, 0.4 μmol/L primer, and 15 μL system for amplification. Three parallel wells were set for each sample. The corresponding upstream and downstream primers were designed and synthesized according to the target gene for PCR amplification. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal reference. Data obtained after three independent experiments were analyzed using the following formula: RQ=2-ΔΔCt. Primer sequences used in this study were as follows: MALAT1, 5'-GGATCCTAGACCAGCATGCC-3' (forward) and 5'-AAAGGTTACCATAAGTAAGTTCCAGAAAA-3' (reverse); GAPDH, 5'-TTCACCACCATGGAGAAGGC-3' (forward) and 5'-GGCATGGACTGTGGTCATGA -3' (reverse)

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 20.0 statistical software (IBM, Armonk, NY, USA) was used for all statistical analysis. Measurement data were expressed as mean ± standard deviation. Independent sample *t*-test was used for comparison between two groups. Count data was expressed by the number of cases and compared by χ^2-test. $p<0.05$ was considered statistically significant.

Table I. Comparison of clinical indicators between death and survival groups.

Variable	Death (n = 63)	Survive (n = 87)	*t*	*p*-value
RBC ($\times 10^{12}$/L)	3.2 ± 0.88	3.5 ± 1.02	1.882	0.062
WBC ($\times 10^{9}$/L)	13.2 ± 6.59	11.6 ± 5.29	1.648	0.102
PLT ($\times 10^{9}$/L)	185.6 ± 89.71	203.4 ± 91.23	1.188	0.237
HB (g/L)	102.1 ± 23.54	112.5 ± 24.59	2.603	0.01
HCT	0.33 ± 0.015	0.45 ± 0.053	17.457	< 0.001
PT (s)	12.6 ± 2.1	11.9 ± 1.68	2.266	0.025
APTT (s)	36.5 ± 3.25	28.4 ± 2.65	16.789	< 0.001
TT (s)	16.5 ± 1.24	18.6 ± 1.49	9.128	< 0.001
DD (mg/L)	7.9 ± 3.28	3.6 ± 1.69	10.467	< 0.001
PaO_2 (mmHg)	54.6 ± 20.81	60.2 ± 21.54	1.594	0.113
$PaCO_2$ (mmHg)	35.2 ± 8.73	36.9 ± 10.52	1.048	0.297
PaO_2/FiO_2 (mmHg)	102.6 ± 46.8	168.7 ± 59.6	7.317	< 0.001
APACHE II Score	29.4 ± 6.28	15.23 ± 5.5	14.668	< 0.001
MODS Score	8.59 ± 3.25	3.58 ± 1.59	12.474	< 0.001

Red blood cell (RBC), white blood cell (WBC), platelet count (PLT), Hemoglobin (HB), hematocrit (HCT), prothrombin time (PT), Activated partial thromboplastin time (APTT), thrombin time (TT), D-dimer (DD), Partial arterial oxygen pressure (PaO_2), partial pressure of carbon dioxide in artery ($PaCO_2$), oxygenation index [(Partial arterial oxygen pressure/inhalation oxygen concentration)] (PaO_2/FiO_2).

Results

Comparison of Clinical Indicators Between Death and Survival Groups

We enrolled 150 elderly patients with severe pneumonia, of which 63 died and 87 survived. Clinical indicators of the two groups were analyzed by *t*-test. The results showed that PT, APTT, DD, APACHE II score, and MODS score were markedly higher in death group than those in survival group ($p<0.05$). However, HB, HCT, TT, PaO_2/FiO_2 showed the opposite trend ($p<0.05$, Table I). This suggested that differences in PT, APTT, DD, HB, HCT, TT, PaO_2/FiO_2, APACHEII scores, and MODS scores might influence the prognosis of elderly patients with severe pneumonia.

MALAT1 Is Highly Expressed in the Serum of Patients in Death Group

We detected MALAT1 expression to explore its impact on patients prognosis. The results demonstrated that MALAT1 was highly expressed in the serum of patients in death group ($p<0.05$, Figure 1).

MALAT1 Evaluates the Sensitivity and Specificity of Patients' Prognosis

ROC curve indicated that the area under the ROC curve (AUC) was 0.7795 ($p=0.0269$, 95% CI=0.7268-0.8323), with a sensitivity of 71.33% and a specificity of 74.67% when cut-off value was 3.20 (Figure 2). The above findings suggested that serum MALAT1 could serve as a potential biomarker for the diagnosis of severe pneumonia in the elderly.

Correlation Between MALAT1 and Clinical Features

To better clarify the correlation between clinical characteristics and MALAT1 expression, according to the cut-off value, we divided patients

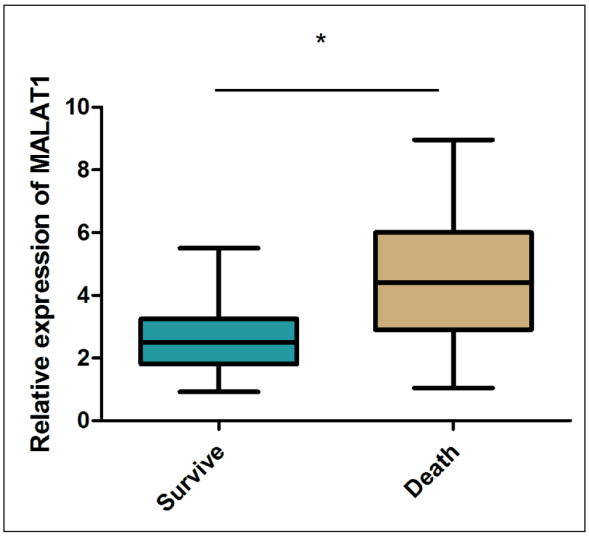

Figure 1. High expression of MALAT1 in the serum of patients in death group. QRT-PCR results showed that the expression of MALAT1 in serum of 63 deaths was significantly higher than that of 87 survivors.

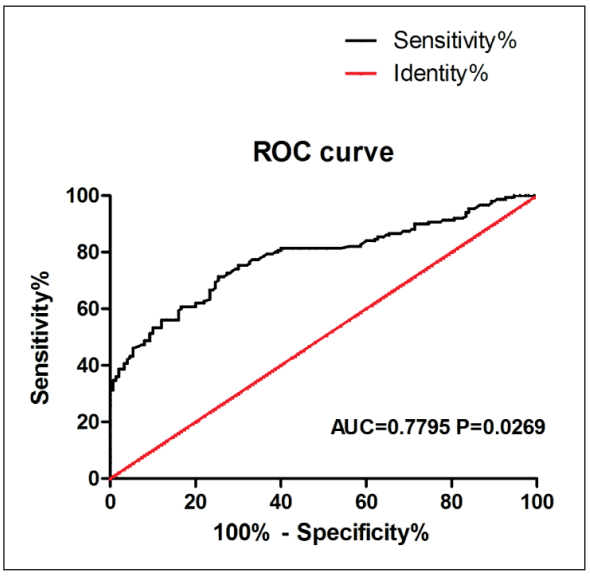

Figure 2. Sensitivity and specificity of MALAT1 assessment of patient prognosis. MALAT1 predicts the prognosis of elderly patients with severe pneumonia. The area under the ROC curve was AUC=0.7795, p=0.0269. When the cut-off value was 3.20, the sensitivity and specificity was 71.33% and 74.67%, respectively.

with MALAT1 below 3.20 into low expression group (n=71) and those with MALAT1 over 3.2 into high expression group (n=79), respectively. The χ^2-test results revealed that MALAT1 increased significantly in patients with a history of smoking and COPD (p<0.05, Table II). These findings further indicated that smoking and the history of COPD might up-regulate the expression of MALAT1.

Discussion

Community-acquired pneumonia (CAP) is the major cause of morbidity and mortality in children and adults worldwide. Meanwhile, it accounts for a large proportion of health-related costs in all health systems. More than 85% patients have a variety of serious underlying lesions or are in a serious disease state[14,15]. Severe pneumonia progresses rapidly, eventually leading to pulmonary physiological dysfunction or even severe organ failure. Furthermore, the mortality of patients with acute respiratory distress syndrome can reach as high as 25%-30% within 28 days[16].

Clinically, several scores have been developed to assess the condition, prognosis, and ICU admission of CAP patients. International Guidelines have also recommended the use of different scores to evaluate the severity and death risk of CAP patients[17]. These scores are mainly related to complications (tumor, liver disease, heart failure, cerebrovascular disease), physiological variables (age, respiratory frequency, urea nitrogen, albumin, etc.) and organ dysfunction (consciousness

Table II. Correlation between MALAT1 and clinical features.

Variable	No.	High level (n = 79)	Low level (n = 71)	χ^2	p-value
Sex					
Male	101	51	50	0.585	0.488
Female	49	28	21		
Age					
< 70	71	39	32	0.277	0.626
≥ 70	79	40	39		
Smoking					
No	61	26	35	4.160	0.047
Yes	89	53	36		
History of COPD					
No	67	27	40	7.430	0.008
Yes	83	52	31		
History of hypertension					
No	77	38	39	0.698	0.418
Yes	73	41	32		
History of diabetes					
No	81	47	34	2.028	0.19
Yes	69	32	37		
Family history of cancer					
No	78	43	35	0.395	0.624
Yes	72	36	36		

disorder, shock, respiratory failure). In theory, by identifying high-risk patients, we can provide them with early intensive monitoring and treatment. However, there are many shortcomings in the overly simplified rating. In fact, most CAP patients die within 30 days due to underlying complications and other factors, independent of pathogen factors, and antibiotic selection[18,19]. The risk of older patients is overestimated because the older group may have scored too high because of age, while the risk of younger patients is underestimated. Hence, the use of these scores to identify patients with high probability of dying within the next 30 days does not necessarily mean that these patients will benefit from more aggressive treatment[20,21]. It is urgent, thus, to search for appropriate laboratory indicators for early diagnosis and prognosis evaluation of CAP.

MALAT1, also known as non-coding nuclear-enriched abundant transcript 2 (NEAT2), is a folded, non-coding RNA with 7 kb in length. MALAT1 is predominantly expressed in the nucleus, which is also highly conserved. Deng et al[22] have shown that MALAT1 is expressed in a variety of tissues and cells in mammals. Initially, it was found significantly overexpressed in tumor tissues, being involved in the regulation of tumor cell proliferation, invasion, migration, and metabolism. Meanwhile, MALAT1 is able to regulate the expression of metabolism-related genes, playing a key role in G1/S and mitosis[23]. The abnormal expression of MALAT-1 in different tumors has been confirmed to be related to the prognosis of patients and can serve as a prognostic marker[24]. In this research, we found that MALAT1 was highly expressed in the serum of patients in death group. MALAT1 exhibited the potential to predict the prognosis of severe pneumonia in the elderly. By analyzing the clinical data and relevant clinical indicators, it was found that smoking and COPD history might have an impact on MALAT1 expression in the serum of elderly patients with severe pneumonia.

Conclusions

Briefly, lncRNA MALAT1 is expected to be used as a molecular marker for the diagnosis and prognosis of severe pneumonia in the elderly. Our findings may lay a new foundation for the clinical study of severe pneumonia and open up new insights.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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