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The function and molecular mechanism of CEP55 in anaplastic thyroid cancer

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Abstract. – OBJECTIVE: The purpose of this study was to determine the role of centrosomal protein of 55 kDa (CEP55) in anaplastic thyroid cancer (ATC) and to further explore the mechanism, which might provide a new molecular marker for treatment of ATC.

PATIENTS AND METHODS: The expression level of CEP55 in clinical cases was tested by fluorescence quantitative Real Time-Polymerase Chain Reaction (qRT-PCR). Also, qRT-PCR assay was performed in different TC cell lines. The relationship between CEP55 expression and clinicopathological characteristics was statistically analyzed. Kaplan-Meier curve and Cox's proportional hazards regression model were performed in survival analysis. Further, Western blot assay was used to analyze the protein expression changes in PI3K/Akt pathway.

RESULTS: The expression level of CEP55 in TC tissues showed a noticeable upgrade, especially in ATC. *In vitro*, CEP55 expression was also increased in four kinds of TC cells, in which, the highest expression was found in ATC (TA-K) cells. The clinicopathological features, including lymph node metastasis, distant metastasis, and prognostic index were found to be correlated with the expression level of CEP55. Besides, the ATC patients with higher expression of CEP55 had a statistically worse overall survival (OS) time. In univariate analyses and multivariate analyses, the CEP55 level was an independent prognosis index of patients with ATC. *In vitro* study, CEP55 protein expression level was significantly reduced in si-CEP55-transfected TA-K cells. Notably, the downregulation of CEP55 could suppress the phosphorylation of PI3K and AKT.

CONCLUSIONS: This study found that CEP55 could promote ATC progression, and PI3K/AKT pathway might be the downstream target of its action. These results provided a new therapeutic direction for the treatment of ATC.

Key Words:
Thyroid carcinoma (TC), Anaplastic thyroid cancer (ATC), Centrosomal protein of 55 kDa (CEP55), PI3K/AKT.

Introduction

Thyroid cancer (TC), the most common one among various cancers in the endocrine system, has a yearly increasing incidence rate in world population[1,2], mainly classified into medullary thyroid carcinoma (MTC), follicular thyroid carcinoma (FTC), papillary thyroid carcinoma (PTC), and anaplastic thyroid cancer (ATC). Among them, FTC and PTC were also known as differentiated thyroid cancer (DTC), with good prognosis, while ATC was infrequent, but it was highly invasive and lethal and progresses rapidly, with a mean survival time of less than 6 months and a 5-year survival rate of less than 10%[3,4]. To this day, there was no standard treatment method for ATC, and existing treatment methods were mainly counterproductive[5,6]. Therefore, it was urgent to identify novel molecular targets in the development of ATC to provide new treatment options for ATC patients.

Centrosomes have been considered important in the studies on tumors for a long time[7], forming spindles to induce the movement of chromosomes to the poles of cells, ultimately leading to the equal distribution of chromatin in two daughter cells. However, centrosomes might have abnormalities during cell division due to various factors, giving rise to abnormal formation of spindles and production of aneuploid genomes in daughter cells[8]. Such abnormalities were im-

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portant factors in the development of tumors. For this reason, centrosomes exerted an extremely important regulatory effect on cell division. As a member of the centrosome-related protein family, centrosomal protein of 55 kDa (CEP55) first discovered in 2005 that modulates the separation of two daughter cells in the end stage of cytokinesis[9,10], of which the regulatory effect was detected by more and more subsequent studies and the regulatory mechanism was improved[11,12]. Furthermore, studies of tumors manifested that CEP55 had a direct or indirect relation to many tumors[13-17]. Hence, studying and exploring the role and possible mechanism of CEP55 in ATC might be conducive to the treatment of ATC.

Patients and Methods

Tissue Samples and Cell Lines

A total of 45 ATC patients diagnosed and operated from June 2017 to December 2019 at China-Japan Union Hospital of Jilin University were analyzed. For comparison, we collected tissue samples from patients with other types of malignant thyroid tumors (PTC, n=10, FTC, n=10 and MTC, n=10) or thyroid adenomas (TA, n=10). Specifically, the ATC tissues and para-cancer normal tissues (more than 5 cm away from the ATC tissues) were resected *via* surgery and pathological examination was performed to ensure that there was no cancer cell infiltration in para-cancer normal tissues. The personal information and detailed clinical data of patients, including gender, age, tumor diameter, lymph node metastasis, distant metastasis, acute symptoms, and prognostic index was shown in Table I. The selection of patients was based on the guideline proposed by the Union for International Cancer Control (UICC). This study was approved by the Ethics Committee of China-Japan Union Hospital of Jilin University. Signed written informed consents were obtained from the patients and/or guardians.

The follow-up was conducted by phone calls or outpatient visit to record the survival of patients. The deadline for follow-up was December 2019. The total survival period was from the date of onset to the date of the final follow-up or death, in months.

Human TC cell lines (FTC-133, TPC-1, TA-K, and TT) together with cell line of normal thyroid gland cell line (Nthy-ori3-1) were purchased from American Type Culture Collection (ATCC; Manassas, VA, USA) and were cultured in the Dulbecco's Modified Eagle's Medium (DMEM) (Gibco, Rockville, MD, USA) containing 10% fetal bovine serum (FBS; Gibco, Rockville, MD,

Table I. Association between CEP55 expression and clinical factors.

Characteristic	CEP55 level		*p*-value
	Positive	Negative	
ATC patients	23	23	
Gender			0.386
Female	10	15	
Male	13	10	
Age (year)			0.554
> 60	12	9	
≤ 60	11	14	
Maximal tumor diameter (cm)			0.136
> 5	16	10	
≤ 5	7	13	
Lymph node metastasis			
Positive	17	8	0.017 *
Negative	6	15	
Distant metastasis			
Positive	10	3	0.047 *
Negative	13	20	
Acute symptoms			
Positive	16	11	0.231
Negative	7	12	
Prognostic index			
0-1	6	18	0.001***
2-4	17	5	

USA) and 1% penicillin-streptomycin. The cells in the logarithmic phase were fetched for transfection.

The si-negative control (si-NC) or si-CEP55 were transiently transfected into TA-K cells using Lipofectamine™ 3000 (Invitrogen, Carlsbad, CA, USA), and the transfected TA-K cells were cultured in the incubator with 5% CO_2 at 37°C. At 48 h after transfection, the cells were collected for subsequent experiments.

Fluorescence Quantitative Real Time-Polymerase Chain Reaction (qPCR) Analysis

The total RNAs were extracted from tissue and cell samples *via* the TRIzol method (Invitrogen, Carlsbad, CA, USA), and reversely transcribed into complementary deoxyribonucleic acids (cDNAs) using random primer method. According to the instructions of a qRT-PCR quantitative detection kit (TaKaRa, Otsu, Shiga, Japan), pre-mixed solution, cDNA template, and corresponding forward and reverse primers of CEP55 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were added for reaction under the following conditions: 95°C for 3 min, 40 cycles of denaturation at 95°C for 15 s, annealing at 60°C for 20 s, and extension at 72°C for 20 s, with fluorescence obtained at 72°C in each cycle. The results of qRT-PCR were processed using $2^{-\Delta\Delta Ct}$ method for quantitative analysis of CEP55. The sequence was shown in Table II.

Western Blots Analysis

The TA-K cells treated were lysed with radioimmunoprecipitation assay (RIPA) lysis buffer (Beyotime, Shanghai, China) and centrifuged at 12000 × g for 15 min, followed by collection of the supernatant. Next, the concentration of proteins was determined, and the supernatant was added with protein loading buffer [5×sodium dodecyl sulfate (SDS)] at a ratio of 4:1, fully heated and boiled for later use. Afterwards, an appropriate volume of samples was taken and separated with 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), followed by wet transfer to a polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA). Thereafter, the membrane was blocked with 3% skimmed milk powder, incubated with primary antibody overnight and corresponding secondary antibody for 1 h, and then, washed. After that, the membrane was placed in an exposure-imaging instrument in a dark room and we added a chemiluminescence substrate to detect the intensity of the chemiluminescence of the target protein bands (Thermo Fisher Scientific, Waltham, MA, USA).

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 19.0 software (IBM Corp., Armonk, NY, USA) was selected for the processing of result data, and measurement data were expressed as mean ± standard deviation ($\bar{x} \pm s$). Paired *t*-test was used to compare the expression level of CEP55. Group χ^2 test was adopted to analyze the associations of the expression of CEP55 in ATC tissues with the clinicopathological features of patients. Overall survival (OS) of the patients was evaluated *via* Kaplan-Meier survival analysis and the intergroup differences were analyzed by Log-rank test. Cox proportional hazard regression model were respectively chosen for single factor analysis and multiple factor analysis of survival analysis. $p<0.05$ suggested that the difference was statistically significant.

Results

CEP55 Expression in Tissue Samples and Cells

In clinical cases, the CEP55 expression was first detected in different type of TC samples. By comparing with adenomas tissues, the expression of CEP55 was found upregulated significantly in

Table II. Sequences.

Gene		Primer sequence
CEP55	Forward primer	5'-GGAGAAAGGAGGGAGCA-3'
	Reverse primer	5'-GATAAACGGAGTGTATTGGT-3'
GAPDH	Forward primer	5'-CCAAGGCTGTGGGCAAGGT-3'
	Reverse primer	5'-GTCGCTGTTGAAGTCAGAGGA-3'

qRT-PCR, quantitative Reverse-Transcription Polymerase Chain Reaction.

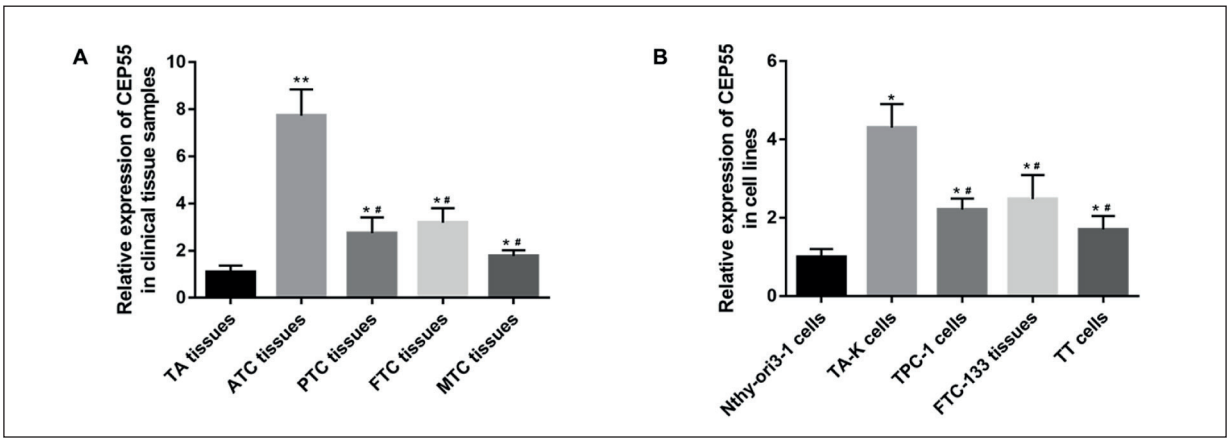

Figure 1. The expression level of CEP55 was measured in clinical tissues (**A**) and cell lines (**B**) by qRT-PCR (*p<0.05, **p<0.01 *vs.* control, #p<0.05 *vs.* ATC).

TC tissues. Of note, we got a higher standard of CEP55 level in the ATC tissues (Figure 1A). In order to learn more about the CEP55 expression in ATC, we analyzed the CEP55 level in different type of TC cell lines. As we expected, the expression of CEP55 *in-vitro* was consisted with that *in-vivo*. ATC cells (TA-K) showed the highest level of CEP55 expression (Figure 1B). Therefore, we considered it meaningful to study the abnormally expressed CEP55 in ATC.

Based on the median expression level of CEP55 from qRT-PCR analysis, we divided ATC tissues into high expression group (n=23) and low expression group (n=23) and the relationship between CEP55 level and clinicopathological features was analyzed. As shown in Table I, the clinicopathological features including lymph node metastasis, distant metastasis and prognostic index were found to be correlated with the expression level of CEP55.

Effect of CEP55 on the Prognosis of Patients with ATC

The correlation between CEP55 level and overall survival time was estimated using the Kaplan-Meier method. As shown in Figure 2, high levels of CEP55 expression indicated a worse prognosis. It is worth noting that after 60 months of follow-up, all survivors were patients with low CEP55 expression.

Univariate Analysis and Multivariate Analysis of CEP55 Expression and ATC Clinicopathological Data

Univariate cox proportional hazards model and multivariate cox proportional hazard model analysis revealed that together with tumor diameter, lymph node metastasis, distant metastasis, acute symptoms, and prognostic index, the expression of CEP55 was also an independent prognosis index. These finding suggested that CEP55 might play an important role in the progression of ATC (Table III).

Effects of CEP55 on PI3K/AKT Pathway

The impacts of CEP55 on the PI3K/AKT pathway were examined through Western blot assay (Figure 3). The results showed that there was an decrease of p-PI3K/total-PI3K and p-ATK/total-AKT in si-NC(−) & si-CEP55(+) group compared with the control group, while no significant change was showed in si-NC(+) & si-CEP55(−) group through comparison with the control group.

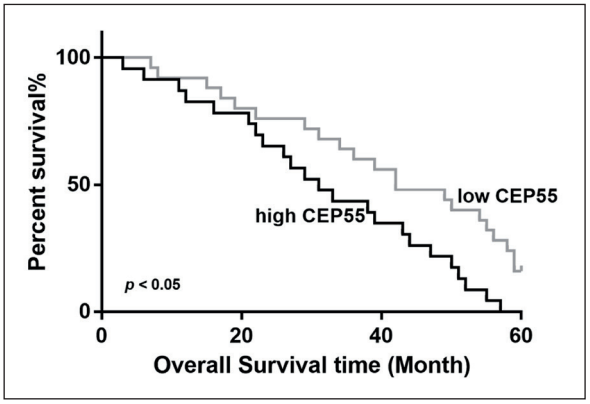

Figure 2. The relationship of CEP55 expression with overall survival of ATC patients (*p<0.05, **p<0.01 and ***p<0.001).

Table III. Correlation between prognosis and patient clinical factors.

Characteristic	Univariate analysis (p-value)	Multivariate analysis (p-value)
Gender	0.421	
Age (year)	0.454	
Maximal tumor diameter (cm)	0.045*	0.049*
Lymph node metastasis	0.013*	0.016*
Distant metastasis	0.002**	0.003**
Acute symptoms	0.014*	0.029*
Prognostic index	0.001***	0.001***
CEP55 expression level	0.038*	0.044*

The results demonstrated that the mechanism of CEP55 in ATC might associated with the activation of PI3K/AKT pathways.

Discussion

ATC is the most malignant thyroid cancer, whose survival rate is much lower than that of DTC. Besides, ATC was a refractory malignant thyroid cancer since it was resistant to radioiodine, with unsatisfactory efficacy of both traditional chemotherapy and radiotherapy. In general, patients died of asphyxia due to local tumor expansion or of distant metastases[18,19]. Thus, finding out the potential therapeutic targets of ATC was a hotspot and a challenge in the current research.

CEP55, an important regulatory protein in surrounding materials of centrosomes, played a vital regulatory role in the assembly of cell

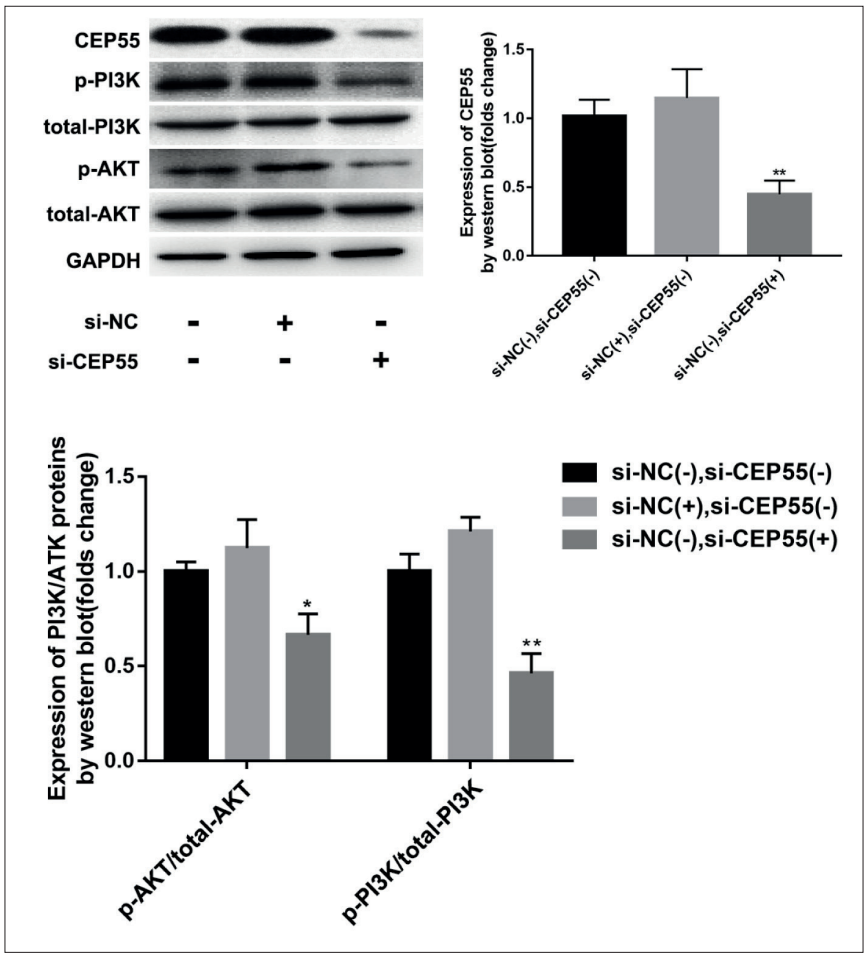

Figure 3. The protein expression of CEP55 and PI3K/AKT-related protein by Western blot (*p<0.05 and **p<0.01).

spindles and the division of cytoplasm. CEP55 is a gene sequence that is located on human chromosome 10q23.33 and contains 9 exons[10], which can couple its C-terminus with CG-Nap and PcntB, microtubule aggregation-related proteins, for multiple phosphorylation to regulate γ-tubulin[20]. Moreover, abnormally expressed CEP55 protein would lead to abnormal structure and function of centrosomes, and the abnormality of centrosomes is common in tumor cells, suggesting that CEP55 might be related to tumorigenesis[21]. Horst and Khanna[22] discovered that the knockdown of the expression level of CEP55 in HELA cells increased the probability of cytokinesis failure, so that most tumor cells failed to undergo cytokinesis, thus resulting in apoptosis. Some scholars[13] have demonstrated that the expression level of CEP55 was significantly higher in bladder transitional cell carcinoma tissues than that in benign tissues, and had an association with various clinical and pathological characteristics of patients. Furthermore, pieces of references manifested that CEP55 was highly expressed in both human gastric cancer and breast cancer tissues and cells, and affected the proliferation of cells[14,15]. Additionally, the high expression of CEP55 was closely correlated with the aggressiveness and prognosis of ovarian epithelial carcinoma[16].

In our research, the expression level of CEP55 was firstly measured *via* qRT-PCR, and it was verified that the expression level of CEP55 was evidently higher in ATC tissues, and it had an association with lymph node metastasis, distant metastasis, and prognostic index. Besides, the survival time of ATC patients with a relatively low CEP55 expression level was remarkably longer than that of those with a relatively high CEP55 expression level, proving that there was a relationship between CEP55 expression level and patient survival. In addition to clinical samples, CEP55 was also expressed differently in diverse cell lines. The expression level of CEP55 was markedly lower in Nthy-ori3-1 cells than that in TC cells. Moreover, the expression level of CEP55 varied in different TC cell lines, which might be related to the diverse cell sources and malignancy degrees. Subsequently, the expression level of CEP55 in ATC cells was knocked down *in vitro* before determination of the expression of PI3K/ AKT pathway-related proteins. The results showed that after knocking down CEP55, the expressions of PI3K and AKT were also affected. For this reason, it was speculated in this study that the mechanism of action of CEP55 in ATC might be mediated by targeting the PI3K/AKT pathway, which was in line with the findings of Chen[23,24]. Other studies pointed out that the effect of CEP55 might be related to the expressions of polo-like kinase 1 (PLK1) and forkhead box protein M1 (FOXM1) proteins. FOXM1 regulated the transcription of PLK1 and CEP55, PLK1 was capable of phosphorylating FOXM1 and CEP55, and CEP55 could promote the expression of FOXM1, so that bidirectional regulation is formed. In addition, CEP55, PLK1, and FOXM1 were inhibited by p53[25-28].

Conclusions

In this study, the comparison of ATC samples with normal adjacent samples and the analysis on the difference in the expression of CEP55 at the tissue level and the cell level. The novelty of this study was that we firstly explored that ATC patients with CEP55 was related to poor prognosis, also, we investigated PI3K/AKT pathway was one of the target pathways by which CEP55 acted on the development and progression of ATC. However, the specific mechanism of CEP55 in affecting the degree of ATC malignancy needed to be further investigated.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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