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ORIGINAL ARTICLE

Expression and clinical value of miR-27a in serum of patients with skin squamous cell carcinoma

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Summary

Purpose: *To investigate the expression and clinical value of miR-27a in the serum of patients with skin squamous cell carcinoma.*

Methods: *70 patients with skin cancer diagnosed and treated in our hospital from July 2015 to July 2018 were selected as the experimental group, and 62 healthy patients with normal physical examination during the same period as the control group. The expression of miR-27a in serum of patients in both groups was detected by fluorogenic quantitative polymerase chain reaction (qRT-PCR). The expression levels of topoisomerase II (topo-II) and human epidermal growth factor receptor 2 (c-erbB-2) were detected by enzyme-linked immunosorbent assay (ELISA). Receiver operating characteristic (ROC) curve was used to analyze the predictive value of miR-27a for poor prognosis and the diagnostic value of miR-27a for skin squamous cell carcinoma.*

Results: *The expression levels of miR-27a, topo-II and c-erbB-2 of patients in the experimental group were significantly higher than those of the control group ($p<0.001$). The 3-year survival rate in the low miR-27a expression group was higher than that of the high expression group. The sensitivity, specificity and AUC of serum miR-27a in predicting the prognosis of skin cancer were 60.71%, 92.86%, 0.765, respectively.*

Conclusions: *The expression of miR-27a in tissue and serum was higher in patients with skin squamous cell carcinoma compared with healthy controls and was closely related to some pathological data of skin cancer. This miR can be used as a detection marker for screening and diagnosing skin squamous cell carcinoma and has a certain predictive value for prognosis.*

Key words: *miR-27a, skin squamous cell carcinoma, topo-II, c-erbB-2, prognosis, diagnosis*

Introduction

Skin cancer is a common malignant tumor with high incidence, mortality, , metastasis and invasiveness [1], and a study has shown that it is related to exposure to sunlight and ultraviolet radiation [2]. Squamous cell carcinoma is the second most common non-melanoma skin cancer [3]. Skin squamous cell carcinoma is superficial and there are many treatment methods, such as surgical resection, radiotherapy, cryotherapy, and laser therapy [4]. If the discovery and treatment are timely and appropriate, the efficacy and prognosis will be better, while if there is regional lymph node metastasis, the prognosis is poor [5]. So early detection plays a decisive role in the diagnosis and treatment of this disease.

Micro RNAs (miRs) are a short-chain non-coding RNA family, which can bind to the 3'UTR region of target genes to regulate their expression. They have important biological functions [6]. As new tumor markers, serum miRs are potential bio-

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markers for the detection of noninvasive cancers [7]. miR-27a, as a member of the miR family, has been found to be related to pathological mechanism of the skin. For example, in the study of Wang et al [8], it was suggested that the upregulation of miR-27a could inhibit the production of melanin in the skin by inhibiting Wnt3a protein. Another study has shown that the level of miR-27a in skin cells is significantly up-regulated under UV stimulation, which may be involved in the coping mechanism of skin in light damage [9]. We studied the protein or gene associated with skin cancer. Among them, topoisomerase II (topo-II) has the function of regulating DNA topology status, which is closely related to cell proliferation, often used as a key target for anticancer drugs. Human epidermal growth factor receptor 2 (c-erbB-2), as an oncogene, is abnormally highly expressed in a variety of human cancers [10,11]. Studies have reported that the level of topo-II in model cell lines of skin cancer (SCC-13, A431) is abnormally up-regulated [12]. Other studies have shown that c-erbB-2 is common in cutaneous squamous cell carcinoma and may be involved in its pathogenesis [13].

At present, there are only few studies on miR-27a, topo-II and c-erbB-2 in skin cancer. This study aimed to investigate their potential clinical significance by detecting the serum expression of the three, expecting to contribute to the clinical diagnosis and prognosis of skin cancer.

Methods

Baseline data

70 patients with skin cancer treated in our hospital from July 2015 to July 2018 and diagnosed by biopsy were selected as the experimental group. There were 48 males and 22 females with an average age of 56.37±3.27 years. Sixty-two healthy volunteers with normal physical examination in our hospital during the same period were selected as the control group. There were 32 males and 30 females with an average age of 58.26±2.07 years. There were no significant differences between the two groups in age, gender, etc.

Inclusion and exclusion criteria

Inclusion criteria: patients who met the diagnostic criteria for skin squamous cell carcinoma [14]; patients who had not received radiotherapy, chemotherapy, and had no other malignant tumors.

Exclusion criteria: patients with liver and renal dysfunction, communication and cognitive dysfunction; patients who did not cooperate with the treating team.

All patients and their families agreed to participate in the experiment and signed the informed consent form. This study was approved by the hospital ethics committees.

Detection method

Before treatment, whole blood was taken and centrifuged at 3000 r/min for 10min at 4℃ to separate plasma. Total RNA was extracted according to the mirVana PARIS kit instructions. RNA was reverse-transcribed into complementary DNA (cDNA). The expression levels of serum miR-27a were detected by real time fluorescence quantitative polymerase chain reaction (real time qRT-PCR). The reaction system was 20 ul SYBR Green I qPCR Master Mix 10 L. There were 0.5 L of upstream and downstream primers, 2 L of cDNA template. The primer sequences are shown in Table 1. The reaction program was: 95 °C for 30 s, 95°C for 10 s, 60°C for 30 s and 72°C for 30 s for a total of 40 cycles. After the reaction, data was collected and the Cycle Threshold (Ct) value of the obtained data was analyzed. U6 was used as the internal reference gene and the relative expression of miR-27a was calculated by the $2^{-\Delta Ct}$ algorithm.

The expression levels of topo-II gene and c-erbB-2 in the serum of each group were determined by enzyme-linked immunosorbent assay (ELISA). 3 ml fasting venous blood was collected from all patients in the morning, and then centrifuged at 3000 r/min for 5 min. After centrifugation, the serum was placed in a refrigerator at -80 °C for subsequent testing. Topo-II and c-erbB-2 levels were detected by ELISA kit. The detection steps were performed according to the instructions.

Experimental materials

Automatic biochemistry analyzer (AU5800, Beckman Coulter, American)
mirVana PARIS kit (Ambion, USA);
miScript Reverse Transcription Kit (Ambion, USA);
Taq-Man MicroRNA Detection Kit (Ambion, USA);
Fluorescence ratio PCR instrument (ABI7900, ABI, USA);
Human C-ErbB-2 Elisa kit (Sciencell, USA, item No. EK0756);
Human TopoII ELISA Kit, (Shanghai Kangjing Bioengineering Co., Ltd., item No. JK--ELISA-00619).

Observation indexes

1. Comparison of baseline data between the two groups.
2. The expression levels of miR-27a in serum and the correlation analysis between miR-27a and topo-II, c-erbB-2.

Table 1. Primer sequences of real time fluorescence quantitative polymerase chain reaction (real time RT PCR)

Genes	*Forward primer 5'-3'*	*Reverse primer 5'-3'*
miR-27a	GCGCTTCACAGTGGCTAAG	GTGCAGGGTCCGAGGT
U6	CTCGCTTCGGCAGCACATATACT	ACGCTTCACGAATTTGCGTGTC

3. The relationship between the expression of miR-27a and the clinical features of patients with skin cancer, such as pathological types, stage, gender, age, differentiation grade, presence or absence of lymph node metastasis, tumor location and tumor thickness.
4. According to the average expression of miR-27a in serum, the patients were divided into high and low expression groups. The Kaplan-Meier survival curve was used to compare the 3-year survival rate of patients with high and low expression. Reactive oxygen species (ROC) curve was used to analyze the prognostic-predictive value of miR-27a in patients with skin squamous cell carcinoma.
5. Drawing a diagnostic ROC curve of miR-27a for skin squamous cell carcinoma.

Statistics

In this study, SPSS 20.0 (Bo Yi Zhixun (Beijing) Information Technology Co., Ltd.) was used for statistical analyses of the experimental data. Chi-square test was used in count data. The measurement data were expressed by mean number ± standard deviation. Independent t-test sample was used to compare measurement data between groups; paired t-test was used to compare values before and after treatment. The Pearson's test was used to analyze the correlation of the indicators. All the pictures in this experiment were drawn using GraphPad Prism 6. Survival analysis was performed by using the Kaplan-Meier and log-rank test. The ROC curve was used to evaluate the value of mir-27a levels in the diagnosis and prognostic prediction of skin cancer. The difference was statistically significant with $p<0.05$.

Results

Comparison of baseline data between the two groups

There was no significant difference in baseline data of age, BMI between the two groups (all $p>0.05$). More details are shown in Table 2.

Table 2. Comparison of baseline data between the two groups (n/%)

Factors	*Experimental group (n=70)*	*Control group (n=62)*	t/x^2	*p*
Age, years	57.37±3.27	58.26±2.07	1.840	0.068
BMI, kg m^2	26.58±3.36	26.14±2.72	0.820	0.414
Gender, n (%)				
Male	48 (68.57)	32 (51.61)		
Female	22 (31.43)	30 (48.39)	3.960	3.961
Hypertension				
Yes	47 (67.14)	35 (56.45)		
No	23 (32.86)	27 (43.55)	0.809	1.598
Diabetes mellitus, n (%)				
Yes	42 (60.00)	33 (53.23)		
No	28 (40.00)	29 (46.77)	0.615	0.961
Hyperlipidemia, n (%)				
Yes	36 (51.43)	34 (54.84)		
No	34 (48.57)	28 (45.16)	0.154	0.997
Smoking, n (%)				
Yes	37 (52.86)	35 (56.45)		
No	33 (47.14)	27 (43.55)	0.171	0.997
Drinking, n (%)				
Yes	38 (54.29)	38 (61.29)		
No	32 (45.71)	24 (38.71)	0.660	0.956
Tumor location, n (%)				
Exposure area, n (%)	47 (67.14)	39 (62.90)		
Genital area, n (%)	23 (32.86)	23 (37.10)	0.260	0.610
Tumor thickness, mm; n (%)				
<2	19 (27.14)	15 (24.19)		
2-5	23 (32.86)	19 (30.65)		
>5	28 (40.00)	28 (45.16)	0.368	0.832

The expression levels of miR-27a in serum of two groups and the correlation analysis between miR-27a and topo-II, c-erbB-2

The expression levels of miR-27a of patients in the experimental group were significantly higher than that of patients in the control group. The difference was statistically significant (all p<0.001). More details are shown in Table 3. The topo-II and c-erbB-2 in the serum of patients in the experimental group were significantly higher than those in the control group and the difference was

Table 3. Expression levels of miR-27a in serum of two groups

Grouping	*n*	*Relative expression*	*t*	*p*
Experimental group	70	1.84±0.76		
Control group	62	0.35±0.23	14.84	<0.001

Table 4. Detection of other related indexes of patients in two groups

Grouping	*Experimental group (n=70)*	*Control group (n=62)*	*t*	*p*
topo-II	1.67±0.23	0.78±0.12	27.34	<0.001
c-erbB-2	8.56±0.22	6.72±0.23	46.94	<0.001

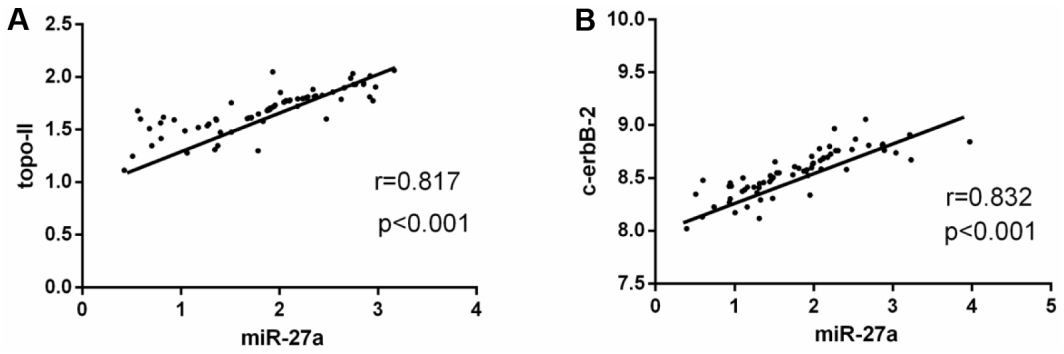

Figure 1. Correlation analysis between miR-27a and the expression of topo-II, c-erbB-2. **A:** miR-27a was positively correlated with the expression of topo-II (r=0.817, p<0.001). **B:** miR-27a was positively correlated with the expression of c-erbb-2 (r=0.832, p<0.001).

Table 5. Relationship between expression of miR-27a and clinical features of patients

Clinical features	*Category*	*n*	*miR-27a*	*t*	*p value*
Gender	Male	48	1.52±0.08	1.240	0.220
	Female	22	1.49±0.12		
Age (years)	≤50	25	1.53±0.07	0.960	0.340
	>50	45	1.51±0.09		
Tumor stage	I/II	34	1.23±0.11	22.28	<0.001
	III/IV	36	1.87±0.13		
Pathological types	Ulcerative type/melanin type	53	1.97±0.15	22.50	<0.001
	Fibrosis type/superficial type	17	1.07±0.12		
Differentiation grade	High/middle	40	1.04±0.12	24.90	<0.001
	Low	30	1.87±0.15		
Lymph node metastasis	Yes	25	1.73±0.14	21.30	<0.001
	No	45	1.02±0.13		
Tumor location	Exposure area	47	1.41±0.12	1.755	0.084
	Genital area	23	1.63±0.15		
Tumor thickness	<2 mm	19	1.34±0.13	29.26	<0.001
	2-5 mm	23	1.47±0.15		
	>5 mm	28	1.73±0.18		

Table 6. Comparison of survival rates of patients with different expression levels of miR-27a

Influencing factors	*n*	*Total deaths n (%)*	*3-year survival rates n (%)*
miR-27a			
High expression group	40	18 (45)	55
Low expression group	30	10 (33.33)	66.67

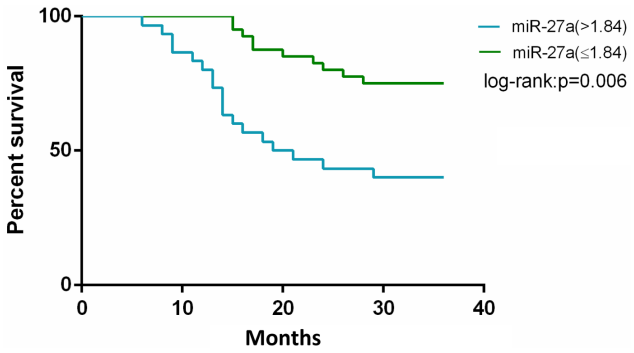

Figure 2. Comparison of survival of patients with different expression levels of miR-27a. All the images in this experiment were plotted using GraphPad Prism 6. Kaplan-Meier survival curves showed that the survival of patients in the high expression group was lower than that in the low expression group (log-rank: p=0.006).

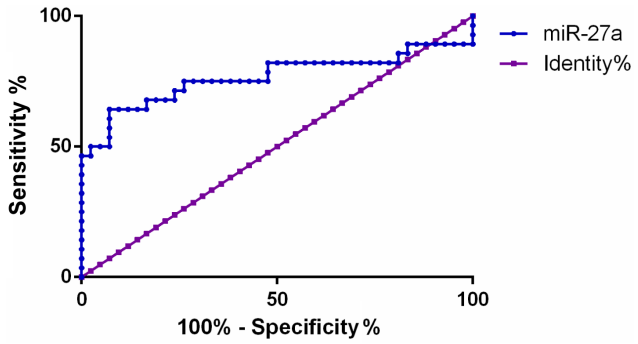

Figure 3. Diagnostic value of miR-27a for poor prognosis in patients with skin squamous cell carcinoma. ROC curve analysis showed that the prognostic diagnostic sensitivity of miR-27a to skin squamous cell carcinoma was 60.71%, the specificity was 92.86%, the AUC was 0.765, the 95% CI was 0.631-0.899 and the cut-off value was 1.952.

statistically significant (p<0.001). More details are shown in Table 4. The expression of miR-27a was positively correlated with the expression of topo-II and c-erbB-2 and the difference was statistically significant (p<0.001). More details are shown in Figure 1.

Relationship between expression of miR-27a and clinical features of patients

The expression of miR-27a was not related to gender, age and tumor location, and the difference was not statistically significant (all p>0.05). But it was related to pathological types, tumor stage, differentiation grade, presence or absence of lymph node metastasis and tumor thickness. The difference was statistically significant (all p<0.001). More details are shown in Table 5.

Comparison of patient survival in the two groups according to miR-27a expression

According to the average expression of miR-27a in the serum of patients, patients were divided into 40 cases in the high expression group and 30 cases in the low expression group. The same therapeutic means were used. The survival rate was evaluated after three years of patient follow-up and was 66.67% in the low expression group, higher than that of the high expression group (55%). More details are shown in Table 6 and Figure 2.

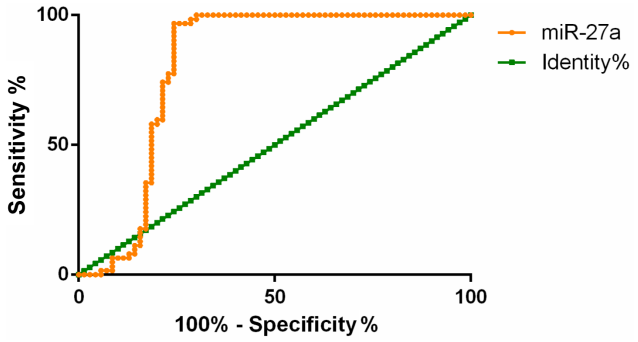

Figure 4. The diagnostic ROC curve of miR-27a for skin squamous cell carcinoma. The diagnostic sensitivity of miR-27a to skin squamous cell carcinoma was 74.29%, the specificity was 96.77%, the AUC was 0.808, the 95% CI was 0.722-0.893 and the cut-off value was 1.081.

Diagnostic value of miR-27a for poor prognosis in patients with skin squamous cell carcinoma

According to whether skin squamous cell carcinoma patients survived for 3 years, patients of the experimental group were divided into 42 cases in the survival group and 28 cases in the death group. The average expression of serum miR-27a of patients in the surviving group and the death group was 1.53±0.21 and 2.34±0.12, respectively. The sensitivity of serum miR-27a in the diagnosis of skin squamous cell carcinoma was 60.71%, the specificity was 92.86%, the AUC was 0.765, the 95%

CI was 0.631-0.899 and the cut-off value was 1.952. More details are shown in Figure 3.

Diagnostic value of miR-27a for skin squamous cell carcinoma

The diagnostic sensitivity of miR-27a in skin squamous cell carcinoma was 74.29%, the specificity was 96.77%, the AUC was 0.808, the 95% CI was 0.722-0.893 and the cut-off value was 1.081. More details are shown in Figure 4.

Discussion

Squamous cell carcinoma is often transformed by keratosis, leukoplakia and other precancerous conditions [15]. The pathogenesis of squamous cell carcinoma is usually related to the proliferation and differentiation of cells. The proliferation and dysplasia of basal cells are closely related to invasion, metastasis and recurrence of tumor [16]. With the study of the pathogenesis of skin squamous cell carcinoma, it is believed that inhibition and apoptosis of cells also plays an important role in the occurrence and deterioration of squamous cell carcinoma [17]. miRs, as highly conserved short RNAs that exist steadily in the body and are widely involved in the physiological and pathological processes of the body, play an important role in cell differentiation, proliferation, apoptosis, migration and other processes. They can also interfere in genes of cancer inhibition or cancer promotion and participate in the occurrence and deterioration of malignant tumors [18]. Among them, miR-27a can play the role of oncogene and lead to the deterioration of condition by regulating processes such as cell apoptosis and cell cycle [19], so it is very important to explore the expression and clinical value of miR-27a in skin squamous cell carcinoma.

In our study, we first compared the expression levels of serum miR-27a in the experimental and control groups. The results showed that the expression levels of miR-27a of patients in the experimental group were significantly higher than that of patients in the control group and indicated that the expression of miR-27a in skin squamous cell carcinoma was higher than that of normal healthy subjects. Studies have shown that miR-27a was highly expressed in breast cancer and participated in the occurrence and deterioration of the disease [20]. Studies have also shown that increased expression of miR-27a is associated with the proliferation and migration of gastric cancer cells [21]. It is speculated that the increased expression of miR-27a is also associated with the occurrence and deterioration of skin squamous cell carcinoma. Topoisomerase II (topo-II) belongs to a class of enzymes in the nucleus [22]. Studies have found that inhibition of topo-II activity can relatively inhibit the rapid proliferation of tumor cells and induce apoptosis and necrosis of tumor cells [23]. Abnormal regulation of c-erbB-2 is generally considered to be an important mechanism of tumor progression and recurrence [24], and studies have confirmed that C-erbB-2 is relatively close to structure of epidermal growth factor receptor [25,26]. This receptor usually shows strong expression levels on the surface of tumor cells. Over-expression of C-erbB-2 is associated with more aggressive tumors and worse prognosis. The results showed that the expression of topo-II and c-erbB-2 oncogene of patients in the experimental group were significantly higher than those of patients in the control group. Therefore, we detected the correlation between miR-27a and topo-II and c-erbB-2 oncogene expression in patients with skin squamous cell carcinoma. The results showed that miR-27a was positively correlated with topo-II and c-erbB-2 oncogene and indicated that the detection of miR-27a expression can effectively determine the condition of skin squamous cell carcinoma. However, the specific pathway affecting the expression of miR-27a and the expression of topo-II and c-erbB-2 oncogene is still unclear, which needs to be further explored in subsequent studies.

Subsequently, we found that the expression of miR-27a was not related to gender and age, but it was related to pathological types, tumor stage, differentiation grade and presence or absence of lymph node metastasis. It was suggested that miR-27a is related to the pathological stage and differentiation grade of skin squamous cell carcinoma and indicated the levels of serum miR-27a can be used as a biomarker for predicting the progression of skin squamous cell carcinoma. Studies have shown that different expression levels of miR-27a directly affect differentiation and proliferation of cancer cells and determine its pathological type and prognosis [27]. Ding et al [28] found that miR-27a activates the downstream AKT/GSK3β signaling pathway by acting on PHLPP2, which ultimately leads to the proliferation and metastasis of gastric cancer. Pan et al [29] found that MAP2K4 is a functional target gene of miR-27a. Inhibition of miR-27a in osteosarcoma cells can increase the protein expression levels of MAP2K4. The proliferation, migration and invasion of MG63 osteosarcoma cells were inhibited by activation of the JNK/p38 signaling pathway. It is speculated that the signaling pathway of miR-27a expression in skin squamous cell carcinoma may also be related to it, but the specific action mechanism remains to be further studied in subsequent experiments.

In this study, patients with skin squamous cell carcinoma were divided into high and low expression groups and they were followed up for 3 years. The survival rate was recorded. The effect of miR-27a expression level and survival rate was obtained and the results showed that the survival rate of the low expression group was significantly higher than that of the high expression group, indicating that the level of miR-27a expression levels has a certain impact on the survival rate of patients. Other studies have confirmed that high level of mir-27a is associated with lower survival rate in prostate cancer patients [30], which is consistent with our study. Subsequently, according to whether patients with skin squamous cell carcinoma survived for 3 years, the experimental group patients were divided into the survival group and the death group. The serum miR-27a expression and prognostic diagnostic value of skin cancer patients were studied. The results showed that the diagnostic sensitivity and specificity were both high. We also studied the diagnostic value of miR-27a in patients with skin squamous cell carcinoma which showed that sensitivity and specificity were also high, indicating that miR-27a can be used as a relative detection marker for screening and diagnosing skin squamous cell carcinoma. Studies have shown [31] that a group of miRs including miR-27a can be used as a sensitivity and specificity tool for diagnosing breast cancer and can be a promising potential biomarker for the diagnosis of breast cancer. It is further illustrated that miR-27a is highly reliable as a biomarker for the diagnosis of several cancers.

Conclusion

The expression levels of miR-27a are significantly increased in patients with skin squamous cell carcinoma and are related to pathological type, tumor stage, differentiation grade and presence or absence of lymph node metastasis. They help monitor the progress and prognosis of the disease in real time. MiR-27a has certain positive significance for the early diagnosis of skin squamous cell carcinoma. However, this study has some limitations. First, the action pathway of miR-27a in squamous cell carcinoma of the skin is still unclear. Second, there was no analysis of the causes of skin cancer, such as radiation and light, so this issue needs to be further explored in subsequent studies.

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Conflict of interests

The authors declare no conflict of interests.

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