Up-Regulation of YTHDF1 and YTHDF3 is Related to the Prognosis of Oral Squamous Cell Carcinoma

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Research

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Abstract

Background

N6-methyladenosine (m6A) is the most abundant internal modification in mammalian mRNAs. M6A is composed of methyl-transferases, which act as ("writers"), binding proteins, which act as ("readers"), and demethylases, which act as ("erasers"). As an important part of ("readers"), YTHDF1 and YTHDF3 were shown to be associated with many cancers. This paper aimed to study the expression of YTHDF1 and YTHDF3 in oral squamous cell carcinoma (OSCC).

Methods

We detected the expression of YTHDF1 and YTHDF3 in 120 OSCC patients by immunohistochemical analysis. Statistical analysis was used to determine whether the high or low expression of these two genes was significantly associated with age, gender, histological type, clinical stage, or lymph node metastasis. The correlation curve and survival curve of the two genes were produced to evaluate the potential clinical significance.

Results

We find the expression of YTHDF1 and YTHDF3 was increased in OSCC tissues compared to adjacent normal tissues□The statistical analysis showed that the expression of YTHDF1 and YTHDF3 was significantly associated with the clinical stage and histological type in OSCC patients. There was also a significant correlation between the expression of YTHDF1 and YTHDF3. A high expression of YTHDF1 and YTHDF3 was related to poor patient prognosis.

Conclusions

Our findings suggest that a high expression of YTHDF1 and YTHDF3 may be related to poor patient prognosis.

Introduction:

OSCC is the most common head and neck malignancy[1]. Although it has a relatively standard treatment protocol, including surgery, radiotherapy, and chemotherapy, the prognosis of OSCC patients is still poor[2]. The 5-year survival rate has not improved significantly. Therefore, it is particularly important to clarify the molecular mechanism behind OSCC and deliver precisely targeted therapy.

M6A is the most abundant internal modification in mammalian mRNAs[3–4]. M6A is dynamically and reversibly regulated by methyl-transferases, which act as writers, binding proteins, which act as readers,

and demethylases, which act as erasers[5]. Despite its functional importance in various physiological events, the role of m6A in chemical carcinogenesis remains largely unknown. M6A is known to regulate gene expression through m6A readers, which is a group of proteins that can recognize the modification in m6A[5]. YTHDF1, YTHDF2, and YTHDF3 are found in the cytoplasm of homologous YT domain family proteins[6]. YTHDF1 promotes the translation of m6A-methylated mRNA, and the translation of YTHDF2 accelerates the decay of m6A-methylated mRNA. YTHDF3, YTHDF2, and YTHDF1 were shown to significantly promote the metabolism of m6A-methylated mRNA[7–9]. The dynamic interplay between m6A pathway components cooperatively regulates the fine-tuning of mRNA metabolism and translation[10]. However, the mechanism of YTHDF1 and YTHDF3 in OSCC has not been established.

Here, we first analyzed the expression of YTHDF1 and YTHDF3 in OSCC and its correlation with clinical and pathological classifications and prognosis.

Materials And Methods:

Patients:

One hundred and twenty cases of OSCC were retrieved from the Department of Oral and Maxillofacial Surgery at Qingdao University between January 2013 and January 2018 by searching the electronic Hospital Information System Clinical and confirming that follow-up data were available for all the study patients. The patients were men and women with an average age of (state the number) years (range 23 – 80). The project was approved by the local ethics committees, and informed consent was obtained from the study patients.

Tissue specimens

Immunohistochemical analysis was performed on the formalin-fixed paraffin-embedded specimens of the clinically and histologically proven OSCC cases and the corresponding adjacent non-tumoral tissues, including 18 stage-I patients, 51 stage-II patients, 23 stage-III patients, and 28 stage-IV patients. Among the selected cases, 83 patients were lymph node-negative, and 37 were lymph node-positive, indicating metastasis. With regard to the histological degree of differentiation, 71 cases were well-differentiated, 29 were moderately differentiated, and 20 were poorly differentiated.

Immunohistochemical staining

The resected OSCC specimens and their corresponding normal tissues were immediately immersed in 4% neutral-buffered formalin, fixed overnight, and embedded in paraffin according to standard procedures. Serial sections (4-mm) cut from representative formalin-fixed, paraffin-embedded cancer tissue onto glass slides were used for immunohistochemical staining. Endogenous peroxidase activity was blocked using 0.3% hydrogen peroxide. After incubation with normal goat serum (ZSGB-BIO), the sections were incubated with anti-YTHDF1 polyclonal antibody and anti-YTHDF-3 antibody (diluted 1:200) overnight at 4•C. The sections were then incubated with biotinylated goat anti-rabbit IgG (ZSGB-

BIO) at room temperature for 30 min. Between each step, the sections were washed with three changes of phosphate-buffered saline (PBS) for 3 min. The reaction product was visualized with DAB stain for 2 min. The tissue sections were then dehydrated with ethanol and xylene, mounted, and coverslipped.

Immunohistochemical analysis

The expression of YTHDF1 and YTHDF3 was examined in five different fields at 20× magnification using a microscope equipped to study the expression. Immunohistochemical signals from 500 cells were evaluated. Distinctly positive cells demonstrated a diffuse brown signal in the cytoplasm, independent of intensity. To eliminate inter-observer bias, the expression of YTHDF1 and YTHDF3 was analyzed independently by two pathologists unaware of the patients' clinical information. Based on the immunostaining scores, the specimens were divided into two groups: those with more than 45% immunopositive cells, which were considered positive for protein overexpression, and those with immunostaining scores of 45% or less, which were considered negative for protein overexpression.

Statistical analysis

Statistical analysis was carried out using SPSS26 software. The chi-squared test was used to examine possible associations between the pathology, immunohistochemical, and clinical results. The Kaplan-Meier method was used to calculate the postoperative survival rate from diagnosis to the occurrence of disease-related death, and to test the difference between the curves. The log-rank test was used to determine statistical significance. Spearman's bivariate correlation was used to analyze the expression of YTHDF1 and YTHDF3 in OSCC tissues. A P-value of < 0.05 was considered statistically significant for all statistical analyses.

Results:

Clinical and pathological features

All 120 patients studied were clinically and histologically proven to have primary OSCC. The patients' ages ranged from 23 to 81 years, and the mean age at primary diagnosis was 59.31 years. Eighty two cases of OSCC occurred in males and 38 in females. The cases were histologically classified into three levels of differentiation. There were 71 well-differentiated, 29 moderately differentiated, and 20 poorly differentiated types. TNM staging was performed according to the American Joint Committee on Cancer (AJCC). Eighteen cases were stage I, 51 were stage II, 23 were stage III, and 28 were stage IV. Eighty-three cases were lymph node-negative, and 37 were lymph node-positive, indicating metastasis. At the last follow-up, which ranged from 3.6 to 136.2 months (median 46 months) and was available for all 120 cases, no additional recurrences were reported and none of the patients underwent chemotherapy.

Immunohistochemical findings

The results of the expression of YTHDF1and YTHDF3 were as follows. The expression of YTHDF1 (Fig. 1a) and YTHDF2 (Fig. 1b) in normal tissues was rarely observed. YTHDF1 was mainly located in the

cytoplasm (Fig. 2), whereas YTHDF3 was expressed in both the cytoplasm and nucleus (Fig. 3). A comparison of the frequency of expression of each histological grade of OSCC showed that the worse the degree of differentiation, the higher the level of expression of both proteins. These results indicated that the overexpression of YTHDF1 and YTHDF3 may cause them to act as oncogenes, involved in the occurrence and development of OSCC.

Patient outcomes

Clinicopathological analysis verified our hypothesis that the expression of both proteins in cancer tissues would increase. We calculated the postoperative survival curves of patients with high and low YTHDF1 and YTHDF3 expression. All 120 follow-up patients had a follow-up period of 100 months. At the completion of the study, 64 (53.3%) patients had died, and 56 (46.7%) patients were alive. The survival rate showed that YTHDF1 and YTHDF3 overexpression was associated with shorter survival and poorer prognosis. The univariate analysis showed that a high expression of YTHDF1 or YTHDF3 was associated with a low survival rate (Table 2).

High expression of YTHDF1 and YTHDF3 is correlated with malignant clinicopathological features in OSCC patients

In a series of OSCC cases with a high expression of YTHDF1 and YTHDF3（Fig. 5), we examined the association between YTHDF1 and YTHDF3 levels and clinicopathological stages in the progression of OSCC. To further define the clinical significance of protein expression levels in the progression of OSCC, we used statistical analysis techniques to examine the correlation between YTHDF1 and YTHDF3 levels and disease stages. Our results showed that the expression of YTHDF1 was significantly correlated with the clinical stage (P = 0.028) and histological classification (P = 0.001). There was no significant association with age (P = 0.183), lymph node metastasis (P = 0.155) and gender (P = 0.870). High YTHDF3 expression was related with clinical stage (P = 0.005) and histological classification (P = 0.013) There was no significant association between age (P = 0.721), lymph node metastasis (P = 0.20), and gender（P = 0.631) (Table1).

Discussion:

Many studies have focused on the potential link between m6A and cancer[4]. The role of m6A modifications may be different in different tumors. Changes in m6A also affect cancer progression, including proliferation, growth, invasion, and metastasis[16]. Proteins contained in the conserved YTH521-b homologous (YTH) domain, including YTHDF1-3 and YTHDC1-2, have m6A-dependent RNA binding activity[6]. Proteins containing the YTH domain are involved in many RNA processes, such as mRNA splicing, nuclear export, translation, and post-transcriptional regulation[15 17]. In recent years, studies have found that the protein containing the YTH domain plays an important role in the process of post-transcriptional modification, regulating the expression of genes related to cancer and other

processes [14]. However, the relationship between YTHDF1, YTHDF3, and OSCC needs to be studied further.

An immunohistochemical method was used to detect the expression of YTHDF1 and YTHDF3 in OSCC tissues. The study showed that there were considerable differences between OSCC tissues and the corresponding adjacent non-tumor tissues and that the expression of YTHDF1 and YTHDF3 was increased significantly in cancer tissues. YTHDF1 was mainly expressed in the cytoplasm. YTHDF3 was expressed in the cytoplasm and also in the nucleus. This was similar to the expression of YTHDF1 and YTHDF3 in breast cancer and OSCC[18] ,indicating that YTHDF1 and YTHDF3 may be contributing factors to the expression of cancer. M6A-related regulatory factors can play a key role in cancer progression by targeting downstream molecules in an m6A-dependent manner. According to previous studies, YTHDF1 could promote the translation of m6A-modified mRNA in the cytoplasm, YTHDF1 silencing could inhibit the expression of c-Myc protein, and the overexpression of YTHDF1 could enhance the expression of c-Myc protein, indicating that YTHDF1 expression promoted c-Myc expression. At the same time, YTHDF1 can directly interact with c-Myc mRNA, which indicates that YTHDF1 regulates the expression of c-Myc mRNA in a certain way[11]YTHDF3 plays a leading role in accelerating the decay of mRNA and can regulate the RNA accessibility of YTHDF1-2. YTHDF3 affects the translation and decay of methylated mRNAs through synergy with YTHDF1 and YTHDF2. YTHDF3 can affect the functions of YTHDF1 and YTHDF2 [12–13], suggesting that YTHDF1 and YTHDF3 may be involved in the activation of some signal pathways in OSCC, and then activate tumor expression.

We also found that the high expression of these two genes was not statistically related to age, gender, or lymph node metastasis. However, it was closely related to the histological type and clinical stage with statistical significance. We also evaluated the effect of YTHDF1 and YTHDF3 on the survival of patients with OSCC. We found that patients with a high expression of YTHDF1 and YTHDF3 had poor survival outcomes. In addition, the results of the univariate analysis showed that YTHDF1 and YTHDF3 were independent risk factors for OSCC. The high expression of YTHDF1 and YTHDF3 was closely related to the poor prognosis of HCC and BRC patients[18-20]. The expression pattern of YTHDF1 and YTHDF3-related regulatory factors may be considered to be prognostic biomarkers. Here, we report for the first time that the high expression of YTHDF1 and YTHDF3 was closely related to poor clinical characteristics such as survival, suggesting that they play potential roles in the progression of OSCC. A comprehensive understanding of the molecular and biological characteristics of YTHDF1 and YTHDF3 will expand our understanding of the role of m6A modifications in tumors, provide potential markers and prognostic evaluation for OSCC, and propose new therapeutic targets.

Conclusions:

Our study revealed differences in the expression of YTHDF1 and YTHDF3 in OSCC and analyzed the relationship between gene expression and clinicopathology and patient prognosis. We found that the expression of YTHDF1 and YTHDF3 was increased in OSCC and played an important role in long-term

patient prognosis. The in-depth study of these two genes may be helpful for a better understanding of OSCC. This study has laid the foundation for further study of the biological mechanisms of OSCC.

Declarations

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Authors contributions

Rui Shi and Shao-ming Li and Ling Gao has designed this research and edited the manuscript ;Ke-qian Zhi and Wen-hao Ren analysed the date.All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors declare no conflict of interest.

Ethical approval

This article does not deal with ethical.Manuscripts containing no individual person’s data in any form (including individual details, images or videos)

Consent for publication

Not applicable

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Tables

Table1 .Relations between YTHDF1 or YTHDF3 expression and clinicopathological parameters

		YTHDF1		*P* value	YTHDF3		*P* value
		H	L		H	L	
Sex							
Male	82	38(46.3%)	44(53.7%)	0.870	34(41.5%)	48(58.5%)	0.631
Female	38	17(44.7%)	21(55.3%)		14（36.8%）	24（63.2%）	
Age (years)							
<60	52	23(44.2%)	29(55.8%)	0.183	20(38.5%)	32(61.5%)	0.721
>60	68	22(32.4%)	46(67.6%)		24(35.3%)	44(64.7%)	
C-stage							
1	18	8(44.4%)	10(55.6%)	0.028	10(55.6%)	8(44.4%)	0.005
2	51	12(23.5%)	39(76.5%)		13(25.5%)	38(74.5%)	
3	23	12(52.2%)	11(47.8%)		14（60.9%）	9（39.1%）	
4	28	12(42.8%)	16(57.2%)		16（57.1%）	12(42.9%)	
Histological classification							
H	71	18(25.4%)	53(74.6%)	0.001	24(33.8%)	47(66.2%)	0.0013
M	29	18(62.1%)	11 (37.9%)		19(66.5%)	10(33.5%)	
L	20	11(55%)	9(45%)		10(50%)	10(50%)	
Metastasis							
Y	37	18(48.6%)	19(51.4%)	0.155	19(51.2%)	18(48.8%)	0.29
N	83	29(34.9%)	54(65.1%)		34(41%)	49(59%)	

P-value <0.05

Table 2.Univariate analysis (log-rank test)

Variable	χ^2	df	P value
YTHDF1	4.094	1	0.0430
YTHDF3	10.12	1	0.0015

P-value <0.05

Figures

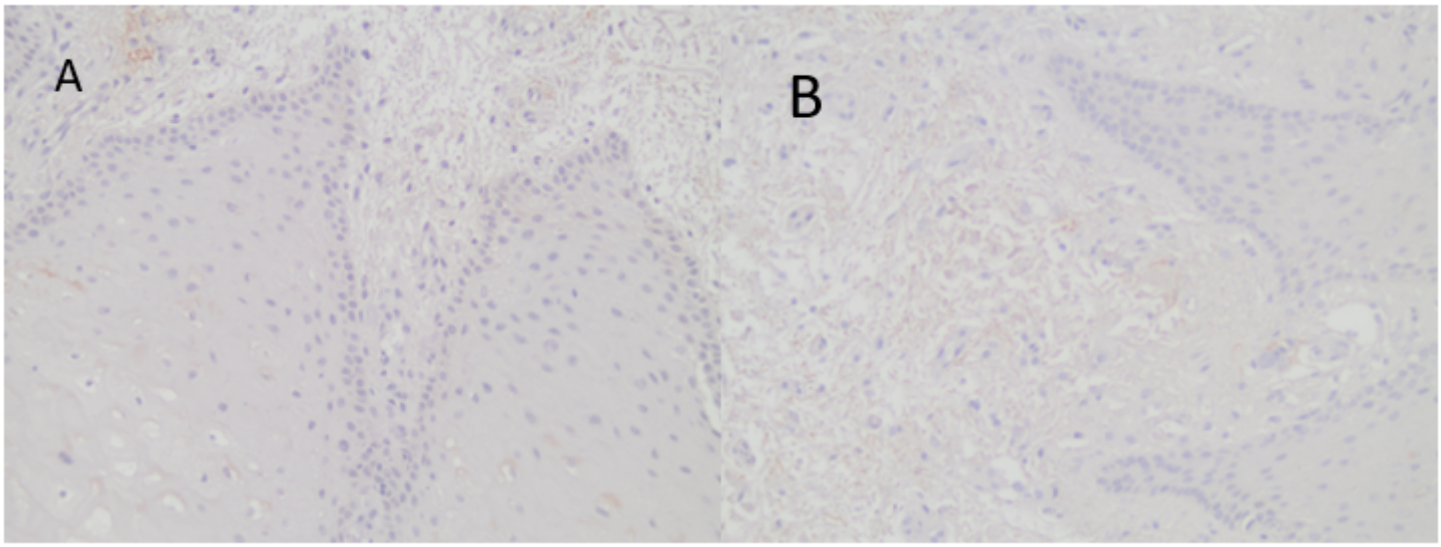

Figure 1

Negative expressions of YTHDF1 (A) and YTHDF3 (B) observed in the basal cells, epithelial cells and lamina propria.

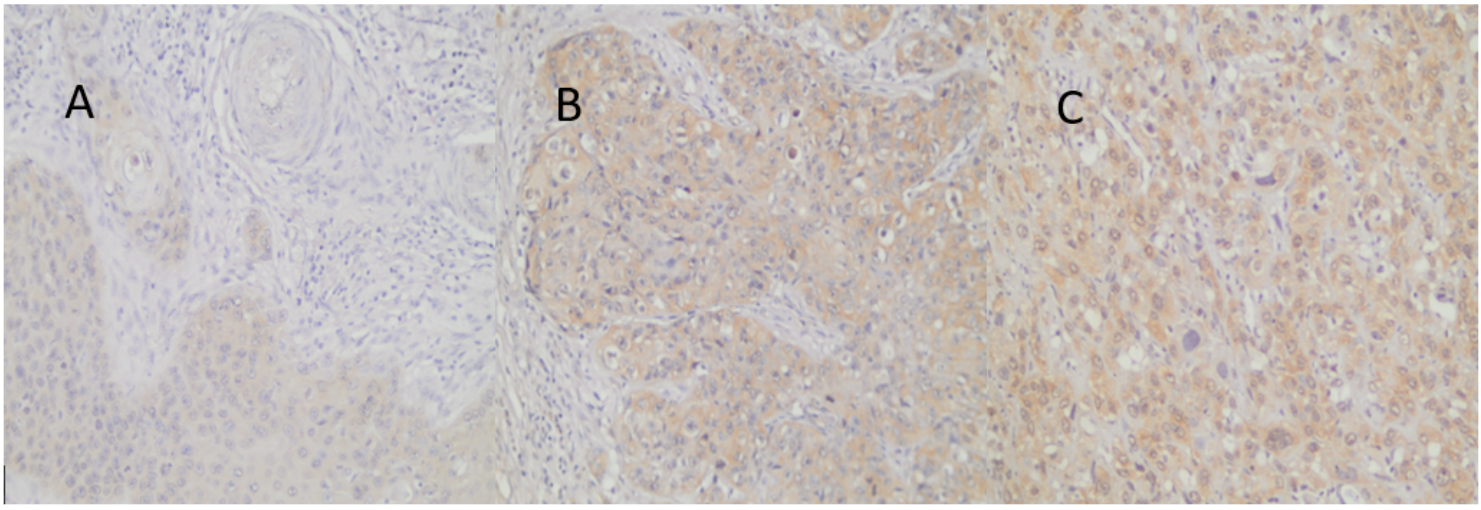

Figure 2

YTHDF1 immunostaining showing expression in cytoplasm of OSCC (A Well differentiated, B moderately differentiated, C poorly differentiated)

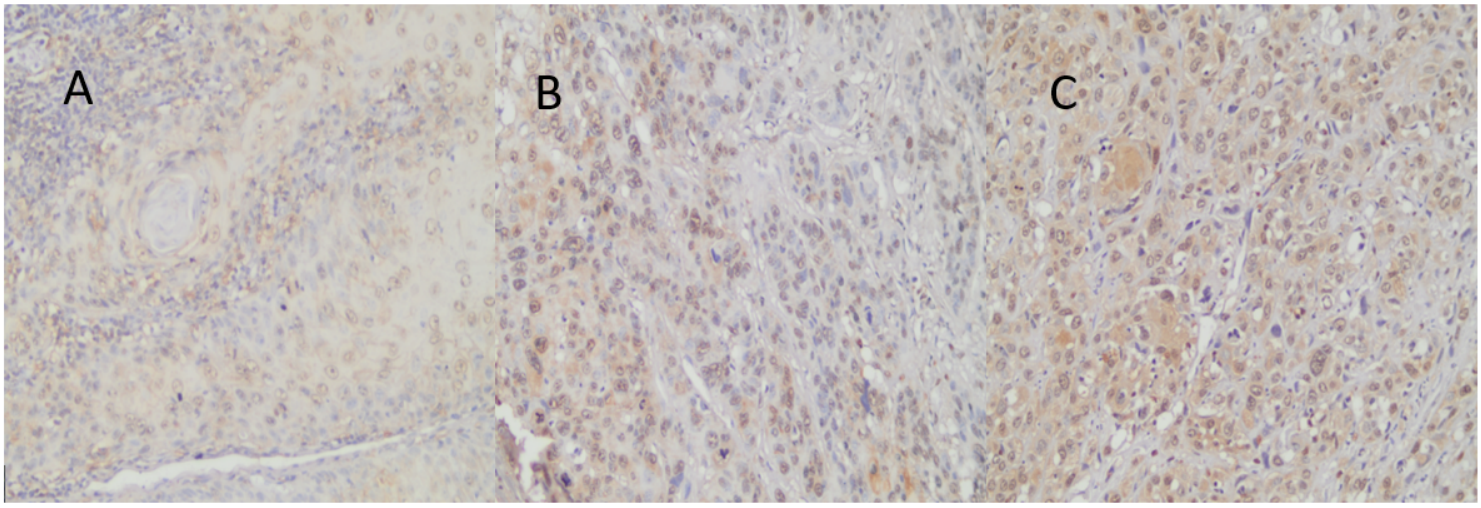

Figure 3

YTHDF3 immunostaining showing expression in cytoplasm of OSCC(A Well differentiated, B moderately differentiated, C poorly differentiated.)

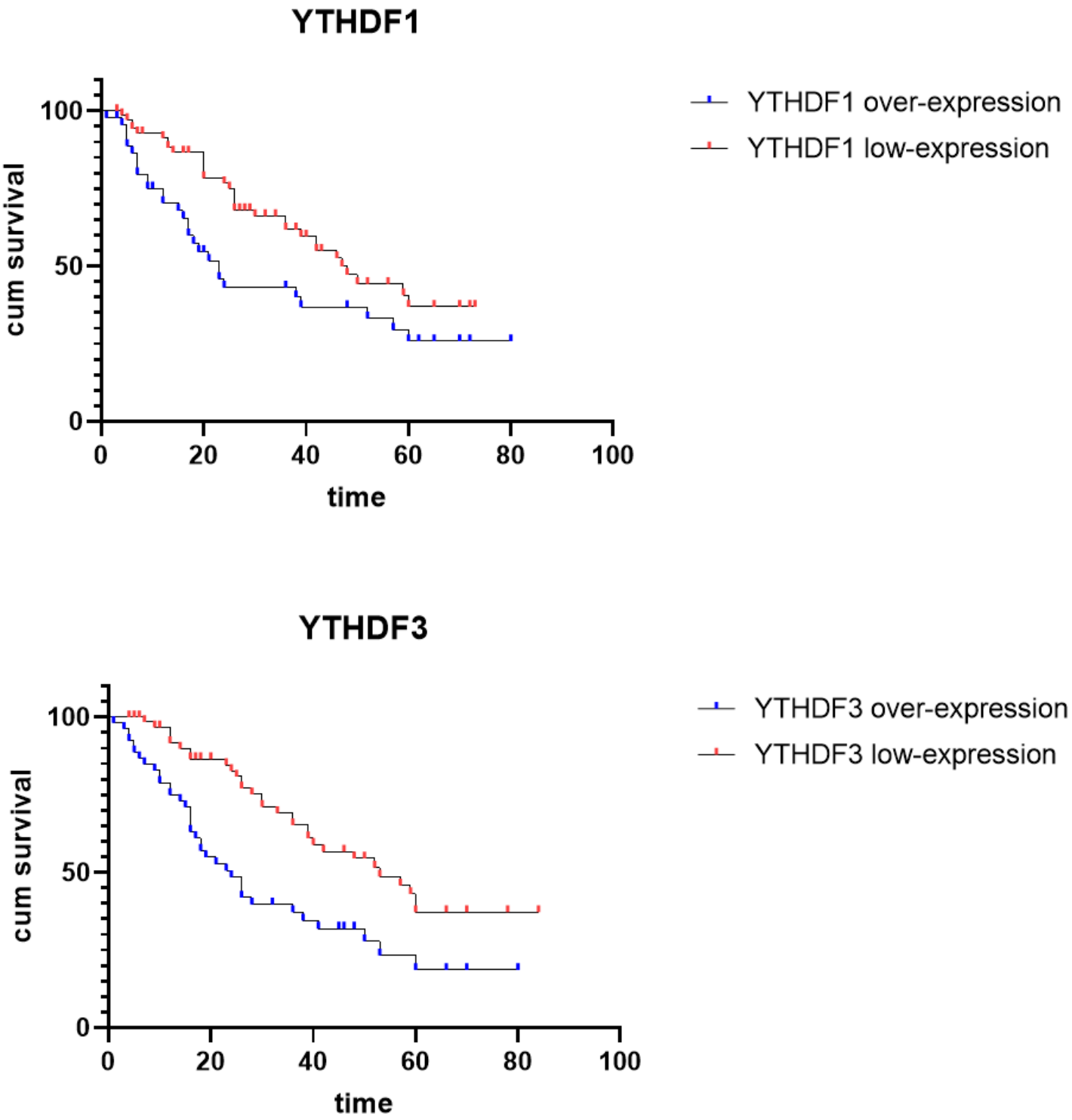

Figure 4

1.YTHDF1 expression and survival outcome for all patients. Survival rates demonstrated that patients with YTHDF1 amplification had a trend towards shorter poor prognosis. 2.YTHDF3 expression and survival outcome for all patients. Survival rates demonstrated that patients with YTHDF3 amplification had a trend towards shorter poor prognosis.

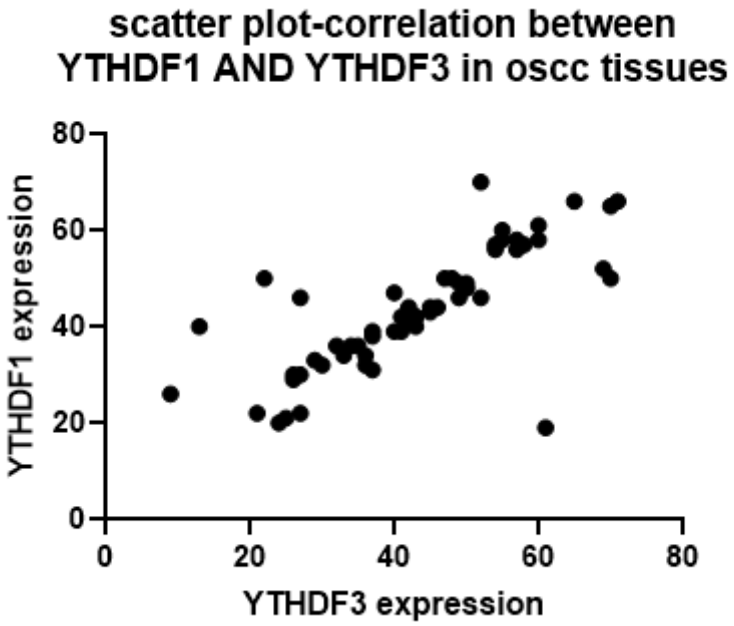

Figure 5

Scatter plot showing there is a correlation between YTHDF1 and YTHDF3 in the OSCC observed.