Clinical and Translational Research

Association and prognostic significance of alpha-L-fucosidase-1 and matrix metalloproteinase 9 expression in esophageal squamous cell carcinoma

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Abstract

BACKGROUND

Alpha-L-fucosidase-1 (FUCA1) has been demonstrated to play opposing regulatory roles in adenocarcinoma and non-adenocarcinoma. Moreover, recent studies reported that FUCA1 could decrease the invasion capability by downregulating matrix metalloproteinase 9 (MMP-9) expression. However, the potential role and prognostic significance of FUCA1 in esophageal squamous cell carcinoma (ESCC) have not yet been explored.

AIM

To evaluate the status, association, and prognostic value of FUCA1 and MMP-9 expression in ESCC.
Esophageal squamous cell carcinoma (ESCC) is the predominantly diagnosed histological subtype in China, accounting for approximately 90% of all esophageal carcinomas (EC) [1]. Although more advances in early screening and multimodal therapy have been approved for use in patients with ESCC, the long-term survival rates even after curative surgery remain unsatisfactory [2-4]. This undesirable prognosis appears to be triggered mostly by aggressive tumor cell invasion and metastasis. Novel modalities for ESCC treatment that target molecular pathways are required to change the prognostic dilemma [5]. In addition, patients diagnosed with
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MATERIALS AND METHODS

The Cancer Genome Atlas data acquisition

The gene expression profiles in tumor tissues, clinical information, survival times, and outcomes of patients diagnosed with ESCC without distant metastasis were obtained from the public The Cancer Genome Atlas (TCGA) database (https://tcga-data.nci.nih.gov/tcga/). The skewed data of FUCA1 and MMP-9 expression profiles were log-transformed to reduce skewness. Then, on the basis of the best cutoff value determined by using X-tile 3.6.3 software (Copyright Yale University 2003), the log-transformed gene expression levels were divided into two groups (high/low).

Patient selection

The clinical, pathological, and follow-up information of all patients who underwent curative esophagectomy for EC from January 1, 2014 to December 31, 2014 at the Sun Yat-Sen University Cancer Center (SYSUCC) was retrospectively collected from the Hospital Information System. All tumor-node-metastasis (TNM) staging was reclassified according to the 8th edition of the American Joint Committee on Cancer Staging Manual. Subsequently, only patients who met the following criteria were retained: (1) Diagnosed with thoracic ESCC; (2) Underwent complete removal (R0); (3) No induction therapy; (4) No death occurred within 30 d after operation; (5) No other primary neoplasm; (6) Had adequate paraffin-embedded specimens for immunohistochemical staining; and (7) Had complete follow-up information. A total of 119 consecutive patients were enrolled in this study (Figure 2).

Written informed consent was obtained from all patients themselves during preoperative conversations. This retrospective study was approved by the Research Ethics Committee at the Sun Yat-Sen University Cancer Center (No. 308–2015–012).

Immunohistochemical staining and interpretation

Detection of FUCA1 and MMP-9 in ESCC tissues was carried out by immunohistochemistry (IHC), as described in related studies [6,8]. Specifically, the paraffin slices were incubated with a rabbit anti-human FUCA1 polyclonal antibody (dilution, 1:400; Abcam, Cambridge, United Kingdom) and a rabbit anti-human MMP-9 monoclonal antibody (dilution, 1:300; D603H, Cell Signaling Technology, Danvers, MA, United...
Figure 1 Schematic diagram of the regulatory mechanisms involved in the effects of alpha-L-fucosidase-1 on malignant tumor invasion and metastasis. MMP: Matrix metalloproteinase; FUCA-1: Alpha-L-fucosidase-1; EGFR: Epidermal growth factor receptor.

States). The expression of FUCA1 and MMP-9 was independently evaluated by two pathologists (Zhang MQ and Huang WT) who all engaged in pathological diagnosis over 5 years. If there was inconsistent interpretation, reevaluation under a double-head microscope was performed to obtain a consistently reliable result.

The staining intensity was graded on a four-step scale: Negative, weak, moderate, and strong, scored as 0, 1, 2, and 3, respectively. The percentage of the chromogenic reaction, counted in five random fields per section using 100 × magnification, was grouped into < 25%, 25%-50%, 50%-75%, and ≥ 75% (scored 1, 2, 3, and 4, respectively). The case was interpreted as high expression of FUCA1 and MMP-9 if moderate to strong cytoplasmic staining was observed in ≥ 25% of ESCC cells with reference to previous studies[13,15]. In addition, the total immunoreaction score was calculated according to the staining intensity (scores 0-3) multiplied by the percentage of stained ESCC cells (scores 1-4) to generate a total score from 0 to 12[13,15].

Statistical analysis
All statistical analyses were performed using SPSS 24.0 software (IBM, Chicago, IL, United States), and a two-sided \( P \) value less than 0.05 was defined as a statistically significant difference. Student’s \( t \) test and \( \chi^2 \) test were used to compare the differences in continuous variables and categorical variables, respectively, between the high expression group and the low expression group. Overall survival (OS) months were counted from the date of ESCC diagnosis to the date of death or the last follow-up (December 31, 2020). The Kaplan-Meier method was applied to identify the potential prognostic variables using the log-rank test. Subsequently, all of the above statistically significant variables were retained in the Cox proportional hazards model. In addition,
scatter plots and survival curves were drawn using GraphPad Prism 8.0 software (San Diego, CA, United States).

RESULTS

Patient characteristics

Of the 119 patients from the SYSUCC, 21 (17.6%) were women, and the mean (standard deviation, SD) age of all patients was 59.0 (8.9) years. The majority of patients were smokers at the time of diagnosis (80, 67.2%) and had a history of alcohol use (74, 62.2%). The middle third of the thoracic esophagus was the most common site of ESCC (69, 58.0%), followed by the lower third (36, 30.3%). Upon pathological examination of resected specimens, the majority of tumors were limited to location between the mucosa and adventitia (pT1, 6.7%; pT2, 16.8%; pT3, 52.1%) under the microscope and had regional lymph node metastasis (pN1, 35.3%; pN2, 10.9%; pN3, 8.4%). The constituent ratios of pathological TNM stages I, II, and III were 5.0%, 39.5%, and 55.5%, respectively. In addition, 67.2% of all patients (80/119) received postoperative adjuvant chemotherapy. Among these 80 patients, 69 (86.3%) received a docetaxel plus nedaplatin/carboplatin regimen, and the remaining 11 were given a paclitaxel plus nedaplatin/carboplatin regimen.

Association of FUCA1 and MMP-9 expression with clinicopathological features

The clinicopathologic features of patients from the SYSUCC and TCGA databases according to the expression status of FUCA1 and MMP-9 are summarized in Table 1 and Supplementary Table 1, respectively. High expression of FUCA1 and MMP-9 in ESCC tumor cells was observed in 90 (75.6%, with a median total score of 6) and 62 patients (52.1%, with a median total score of 3), respectively. The IHC staining for FUCA1 and MMP-9 is shown in Figure 3. High FUCA1 expression was more frequent in patients with positive regional lymph node metastasis ($P = 0.003$, Figure 4A) and advanced stage tumors ($P = 0.029$). In the high MMP-9 expression group, patients showed a lower proportion of smoking history (56.5% vs 78.9%, $P = 0.009$) and alcohol use (53.2% vs 71.9%, $P = 0.036$). The FUCA1 expression status and total score were positively related to the MMP-9 expression status ($P = 0.002$) and total score ($r = 0.258$, $P = 0.005$, Figure 4B), respectively. However, in TCGA data analysis, we only found that male patients ($P = 0.035$) and patients without lymph node metastasis ($P = 0.050$) had a relatively higher proportion of low MMP-9 gene expression (Supplementary Table 1).

Survival outcomes and prognostic analysis

In the SYSUCC cohort, the median OS time was 32.2 mo (range, 1-73 mo) and the 5-year OS rate was 39.5%. In univariate survival analysis, no regional lymph node metastasis (N0, $P = 0.002$), earlier TNM stage (I-II, $P = 0.001$), low FUCA1 expression ($P$
Table 1 Association of alpha-L-fucosidase-1 and matrix metalloproteinase 9 expression with clinicopathological characteristics in patients with resected esophageal squamous cell carcinoma from Sun Yat-Sen University Cancer Center

| Variable                      | FUCA1 expression | MMP-9 expression |
|-------------------------------|------------------|------------------|
|                               | Low expression (n = 29) | High expression (n = 90) | P value | Low expression (n = 57) | High expression (n = 62) | P value |
| Age (yr, mean ± SD)           | 61.2 ± 7.6       | 58.3 ± 9.2       | 0.137    | 59.5 ± 8.4       | 58.6 ± 9.4       | 0.587    |
| Female, n (%)                 | 3 (10.3)         | 18 (20.0)        | 0.236    | 6 (10.5)         | 15 (24.2)        | 0.058    |
| History of smoking, n (%)     | 6 (20.7)         | 33 (36.7)        | 0.111    | 45 (78.9)        | 35 (56.5)        | 0.009*   |
| History of alcohol use, n (%) | 10 (34.5)        | 35 (38.9)        | 0.670    | 41 (71.9)        | 33 (53.2)        | 0.036    |
| Localization, n (%)           |                  |                  |          |                  |                  |          |
| Upper                         | 2 (6.9)          | 12 (13.3)        | 0.360    | 5 (8.8)          | 9 (14.5)         | 0.555    |
| Middle                        | 20 (69.0)        | 49 (54.4)        |          | 33 (57.9)        | 36 (58.1)        |          |
| Lower                         | 7 (24.1)         | 29 (32.2)        |          | 19 (33.3)        | 17 (27.4)        |          |
| Grade, n (%)                  |                  |                  |          |                  |                  |          |
| Well                          | 4 (13.8)         | 19 (21.1)        | 0.648    | 12 (21.1)        | 11 (17.7)        | 0.820    |
| Moderately                    | 16 (55.2)        | 48 (53.3)        |          | 31 (54.4)        | 33 (53.2)        |          |
| Poorly                        | 9 (31.0)         | 23 (25.6)        |          | 14 (24.6)        | 18 (29.0)        |          |
| T stage, n (%)                |                  |                  |          |                  |                  |          |
| T1-2                          | 6 (20.7)         | 22 (24.4)        | 0.678    | 12 (21.1)        | 16 (25.8)        | 0.541    |
| T3-4a                         | 23 (79.3)        | 68 (75.6)        |          | 45 (78.9)        | 46 (74.2)        |          |
| Lymphatic invasion, n (%)     |                  |                  |          |                  |                  |          |
| Negative (N0)                 | 20 (69.0)        | 34 (47.8)        | 0.003*   | 31 (54.4)        | 23 (37.1)        | 0.067    |
| Positive (N+)                 | 9 (31.0)         | 56 (62.2)        |          | 26 (45.6)        | 39 (62.9)        |          |
| TNM stage, n (%)              |                  |                  |          |                  |                  |          |
| I-II                          | 18 (62.1)        | 35 (38.9)        | 0.029*   | 29 (50.9)        | 24 (38.7)        | 0.182    |
| III                           | 11 (37.9)        | 55 (61.1)        |          | 28 (49.1)        | 38 (61.3)        |          |
| Postoperative complication, n (%) |              |                  |          |                  |                  |          |
| Arrhythmia                    | 5 (17.2)         | 22 (24.4)        | 0.421    | 16 (28.1)        | 11 (17.7)        | 0.179    |
| Pneumonia                     | 1 (3.4)          | 11 (12.2)        | 0.172    | 5 (8.8)          | 7 (11.3)         | 0.649    |
| Anastomotic leak              | 0 (0)            | 11 (12.2)        | 0.048*   | 4 (7.0)          | 7 (11.3)         | 0.421    |
| Adjuvant therapy, n (%)       | 19 (65.5)        | 61 (67.8)        | 0.822    | 36 (63.2)        | 44 (71.0)        | 0.365    |
| FUCA1 total score (mean ± SD) | -                | -                | 4.3 ± 3.8 | 7.3 ± 4.1 | 0.000* |
| High FUCA1 expression, n (%)  | -                | -                | 36 (63.2) | 54 (87.1) | 0.002* |
| MMP-9 total score (mean ± SD) | 3.6 ± 2.7        | 2.3 ± 2.6        | 0.029*   | -             | -             | -        |
| High MMP-9 expression, n (%)  | 8 (27.6)         | 54 (60.0)        | 0.002*   | -             | -             | -        |

*P < 0.05.

FUCA1: Alpha-L-fucosidase-1; MMP-9: Matrix metalloproteinase-9; SD: Standard deviation.

= 0.001), and low MMP-9 expression (P = 0.002) were significantly associated with a favorable OS (Table 2). After further adjustment in the Cox proportional hazards model, the above four variables still had strong prognostic value for OS (Table 2 and Figure 5).
Table 2 Kaplan-Meier analysis and Cox multivariate regression analyses of overall survival in patients with resected esophageal squamous cell carcinoma from Sun Yat-Sen University Cancer Center

| Variable                        | No. | Univariate analysis | Multivariate analysis |
|---------------------------------|-----|---------------------|-----------------------|
|                                 |     | 5-yr overall survival rate (%) | P value | Hazard ratio (95% confidence interval) | P value |
| **Age (yr)**                    |     |                     |           |                                       |
| < 60                            | 61  | 44.3                | 0.133     |                                       |
| ≥ 60                            | 58  | 34.5                |           |                                       |
| **Sex**                         |     |                     |           |                                       |
| Female                          | 21  | 28.6                | 0.510     |                                       |
| Male                            | 98  | 41.8                |           |                                       |
| **History of smoking**          |     |                     |           |                                       |
| Yes                             | 39  | 42.5                | 0.533     |                                       |
| No                              | 80  | 33.3                |           |                                       |
| **History of alcohol use**      |     |                     |           |                                       |
| Yes                             | 74  | 40.5                | 0.831     |                                       |
| No                              | 45  | 37.8                |           |                                       |
| **Localization**                |     |                     |           |                                       |
| Upper                           | 14  | 21.4                | 0.426     |                                       |
| Middle                          | 69  | 42.0                |           |                                       |
| Lower                           | 36  | 41.7                |           |                                       |
| **Grade**                       |     |                     |           |                                       |
| Well                            | 23  | 43.5                | 0.222     |                                       |
| Moderately                      | 64  | 43.8                |           |                                       |
| Poorly                          | 32  | 28.1                |           |                                       |
| **T stage**                     |     |                     |           |                                       |
| T1-2                            | 28  | 39.3                | 0.890     |                                       |
| T3-4a                           | 91  | 39.6                |           |                                       |
| **Lymphatic invasion**          |     |                     |           |                                       |
| Negative (N0)                   | 54  | 55.6                | 0.002³    | 0.584 (0.351-0.972)                    | 0.039³  |
| Positive (N+)                   | 65  | 23.5                |           | Reference                              |
| **TNM stage**                   |     |                     |           |                                       |
| I-II                            | 53  | 54.7                | 0.001³    | 0.520 (0.316-0.856)                    | 0.010³  |
| III                             | 66  | 27.3                |           | Reference                              |
| **Arrhythmia**                  |     |                     |           |                                       |
| Yes                             | 27  | 29.6                | 0.210     |                                       |
| No                              | 92  | 42.4                |           |                                       |
| **Pneumonia**                   |     |                     |           |                                       |
| Yes                             | 12  | 33.3                | 0.110     |                                       |
| No                              | 107 | 40.2                |           |                                       |
| **Anastomotic leak**            |     |                     |           |                                       |
| Yes                             | 11  | 36.4                | 0.295     |                                       |
| No                              | 108 | 39.8                |           |                                       |
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| Adjuvant therapy |       |     |      |
|------------------|-------|-----|------|
| Yes              | 80    | 33.7| 0.159|
| No               | 39    | 51.3|      |

| FUCA1 expression |       |     |      |
|------------------|-------|-----|------|
| Low              | 29    | 65.5| 0.001* |
| High             | 90    | 31.1| Reference |

| MMP-9 expression |       |     |      |
|------------------|-------|-----|------|
| Low              | 57    | 56.1| 0.002* |
| High             | 62    | 24.2| Reference |

\*P < 0.05.

FUCA1: Alpha-L-fucosidase-1; MMP-9: Matrix metalloproteinase-9.

Figure 3 Immunohistochemical staining for alpha-L-fucosidase-1 and matrix metalloproteinase 9 in esophageal squamous cell carcinoma. A: Stage IA esophageal squamous cell carcinoma (ESCC) with negative expression of alpha-L-fucosidase-1 (FUCA1) (total score, 0); B: Stage IIB ESCC with low expression of FUCA1 (total score, 2); C: Stage IIB ESCC with high expression of FUCA1 (total score, 12); D: Stage IB ESCC with negative expression of matrix metalloproteinase 9 (MMP-9) (total score, 0); E: Stage IB ESCC with low expression of MMP-9 (total score, 3); F: Stage IIIA ESCC with high expression of MMP-9 (total score, 12). Original magnification: 100×; scale bar: 100 μm.

Similarly, after univariate and multivariate analyses, we found that nonmetastatic ESCC patients (I-III) with low FUCA1 gene expression in the TCGA database were also significantly related to a better OS (\(P = 0.006\); Supplementary Table 2 and Figure 6A). However, the relationship of low MMP-9 expression with favorable prognosis was observed as a trend but with no statistical significance (\(P = 0.080\); Supplementary Table 2 and Figure 6B).

DISCUSSION

In this study, we detected FUCA1 protein overexpression in most ESCC tissues by IHC for the first time and found that high FUCA1 expression was positively associated with high MMP-9 expression, regional lymph node metastasis (pN+), and advanced TNM stage (III). Additionally, multivariable survival analysis showed that the FUCA1 and MMP-9 expression status could independently predict the postoperative survival of patients with resected ESCC.
In 2015, Tzu-Chun Cheng and his colleagues reported that upregulation of FUCA1 expression in triple-negative breast cancer (adenocarcinoma) conferred a favorable OS by degrading cell surface glycoproteins and glycolipids to inhibit cell growth and induce cell death[8]. Subsequently, the association of higher FUCA expression (≥ 20.85 U/L) with a better prognosis in patients with intrahepatic cholangiocarcinoma (adenocarcinoma) was also confirmed. Further mechanistic investigations revealed that FUCA could diminish the invasive ability of tumor cells by downregulating MMP-9 expression[9]. In addition, another recent study on thyroid cancer demonstrated that FUCA1 expression was higher in normal thyroids and papillary thyroid carcinomas than in poorly differentiated, metastatic, and anaplastic thyroid carcinoma; in other words, lower FUCA1 expression was related to a worse prognosis of thyroid cancer (adenocarcinoma)[15]. Similarly, Otero-Estévez et al[16] reported that the expression and activity of FUCA1 in colorectal cancer (adenocarcinoma) showed a gradual decrease from early to advanced stage, and patients with a low FUCA1 level were significantly associated with a higher tumoral recurrence rate. However, there were controversial reports concerning the relationship between FUCA1 expression and prognostic survival in non-adenocarcinoma malignancies (i.e., hepatocellular carcinoma, glioma, and ESCC)[5,7,10,17]. Two early studies reported that a high preoperative serum FUCA level (> 35/μL) was significantly associated with a worse recurrence-free survival and OS in patients with hepatocellular carcinoma following hepatectomy[7,17]. Recently, another study also found that FUCA overexpression had a negative effect on the prognosis of glioma[10]. The prognostic roles of FUCA1 overexpression in hepatocellular carcinoma and glioma were in agreement with our findings in ESCC. Through further mechanistic studies, they elaborated the following novel mechanisms. First, the lack of FUCA1 protein could promote the development of numerous acidic vacuoles that participate in the autophagic cell death process. Second, FUCA1 overexpression induced tumor-associated macrophage recruitment by upregulating chemokines 2/5 expression, but this pathway could be inhibited by introducing FUCA1 silenced RNA[10]. Moreover, correlation analyses showed that FUCA1 overexpression was associated with higher proportions of local invasion, higher pathological grade, and lymphatic metastasis[7,10,17]. The above evidence from mechanistic studies and retrospective cohort studies all illustrated that FUCA1 may have the ability to promote tumor cell invasion and metastasis among patients with non-adenocarcinoma[7,10,17]. Therefore, FUCA1 may be used not only as a prognostic biomarker but also as a novel therapeutic target for hepatocellular carcinoma, glioma, and ESCC. In addition, on the basis of the above studies, it became apparent that the molecular mechanisms of FUCA1 were quite different in adenocarcinoma and non-adenocarcinoma malignancies, and further studies are warranted to elucidate the potential molecular pathways in ESCC.

Figure 4 Correlation analysis. A: Positive lymph node metastasis (N+) is associated with a high alpha-L-fucosidase-1 (FUCA1) total score; B: Low FUCA1 expression is associated with a low matrix metalloproteinase 9 total score. FUCA1: Alpha-L-fucosidase-1.
Many studies observed MMP-9 overexpression detected by IHC staining in ESCC (34.8%-90.0%), and the related mechanistic studies elaborated that MMP-9 could participate in the development and progression of ESCC mainly by degrading type IV collagen to promote tumor cell metastasis and resulted in poor prognosis, which was completely consistent with our present study\textsuperscript{[11]}. However, few studies have focused on the association between FUCA1 and MMP-9 expression. Shuang \textit{et al}\textsuperscript{[9]} reported that AFU (another abbreviation form for FUCA1) significantly downregulated the expression of MMP-9 in intrahepatic cholangiocarcinoma (adenocarcinoma) \textit{in vitro}. Another cytological experiment carried out by Yuan \textit{et al}\textsuperscript{[14]} also demonstrated that AFU could significantly reduce MMP-9 activity and expression in human breast cancer.
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conversely, our study found that FUCA1 staining was positively associated with MMP-9 staining in ESCC, which was first reported in squamous cell carcinoma. The detailed molecular mechanisms need to be explored in the future to obtain more effective therapeutic targets in ESCC.

Although our present study clarified the prognostic significance of FUCA1 expression and the correlation of FUCA1 and MMP-9 expression in ESCC for the first time, several limitations cannot be ignored when extrapolating these results. First, this retrospective study was carried out in a single-center, small sample size cohort, which inevitably caused selection bias. Moreover, external validation in other patient cohorts was also lacking. Second, due to intratumoral heterogeneity, the paraffin section used for IHC staining may not represent the entire tumor mass. To obtain the most representative staining, all paraffin slices used in this study included ESCC tissues and corresponding normal esophageal tissues without necrotic tissue. Third, the antibodies selected in this study were not completely the same as those used in previous studies [12,13,15,18]. Undeniably, different antibodies may vary the IHC staining results. Fourth, the consensus cutoff values for FUCA1 and MMP-9 staining interpretation had not been determined; thus, there may be variation in the statistical results in which different cutoff values were used. In this study, the threshold value was selected for interpreting high and low FUCA1/MMP-9 expression with reference to previous large cohort studies [12,13,15,18].

CONCLUSION

FUCA1 cooperation with MMP-9 may have a major role in affecting the ESCC invasion and metastatic capability and serve as a valuable prognostic biomarker in ESCC.

ARTICLE HIGHLIGHTS

Research background
Fundamental studies discovered that alpha-L-fucosidase-1 (FUCA1) could downregulate matrix metalloproteinase 9 (MMP-9) expression and activity, thereby diminishing the invasive ability of intrahepatic cholangiocarcinoma and triple-negative breast cancer; thus, high FUCA1 expression and low MMP-9 expression in serum or tumor tissue are associated with a favorable prognosis. However, likely due to molecular mechanistic heterogeneity, some studies have reported that elevated FUCA1 content predicts worse survival outcomes in hepatocellular carcinoma and glioma.

Research motivation
To explore the prognostic significance of FUCA1 and MMP-9 expression in esophageal squamous cell carcinoma (ESCC) and investigate the correlation of FUCA1 expression with MMP-9 expression.

Research objectives
A total of 119 consecutive patients who underwent esophagectomy for ESCC between January 1, 2014 and December 31, 2014 at the Sun Yat-Sen University Cancer Center (SYSUCC) were enrolled in the final analysis. In addition, the FUCA1 and MMP-9 gene expression profiles of 76 patients diagnosed with ESCC without distant metastasis were obtained from the public The Cancer Genome Atlas (TCGA) database.

Research methods
Student’s t test and χ² test were used to compare the differences in continuous variables and categorical variables, respectively. The Kaplan-Meier method was applied to identify the potential prognostic variables using the log-rank test. Subsequently, all of the above statistically significant variables were retained in the Cox proportional hazards model.

Research results
In the SYSUCC cohort, the FUCA1 expression status (high/low) and total IHC score were positively related to the MMP-9 expression status (high/low, $P = 0.002$) and total IHC score ($r = 0.258$, $P = 0.005$). Moreover, after further adjusting in the Cox propor-
tional hazards model, low FUCA1 expression ($P = 0.001$) and low MMP-9 expression ($P = 0.002$) still showed significant associations with a favorable overall survival (OS). Similarly, after univariate and multivariate analysis, we found that nonmetastatic ESCC patients (I-III) with low FUCA1 gene expression in the TCGA database also had a significantly better OS ($P = 0.006$). However, the relationship of low MMP-9 expression with a favorable prognosis was observed as a trend but with no statistical significance ($P = 0.080$).

**Research conclusions**

FUCA1 cooperation with MMP-9 may have a major role in affecting ESCC invasion and metastasis capability and serve as a valuable prognostic biomarker in ESCC.

**Research perspectives**

The present study offers a future research direction in which FUCA1 cooperation with MMP-9 may be a potential regulator in ESCC progression, which needs to be explored in fundamental studies.

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