Original Article

Deregulation of decorin and FHL1 are associated with esophageal squamous cell carcinoma progression and poor prognosis

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Abstract: To investigate the expression of FHL1 and Decorin in esophageal squamous cell carcinoma (ESCC) and its clinical significance. 82 ESCC tissues were evaluated by immunohistochemistry for the expression of FHL1 and Decorin. The role of the expression of FHL1 and Decorin in ESCC were statistically analyzed. The expression level of FHL1 and Decorin were reduced in malignant tissue samples in comparison to normal matched tissue (P < 0.05). It was also proved that the positive expressions of FHL1 and Decorin were associated with ESCC histological grade, lymph node metastasis, tumor stage and clinical stage (P < 0.05). In addition, the Kaplan-Meier survival curves revealed that the positive expressions of FHL1 and Decorin were associated with favorable prognosis in ESCC patients. Multivariate analysis showed that the positive expressions of FHL1 and Decorin were independent prognostic markers of overall survival of ESCC patients, respectively. The positive expressions of FHL1 and Decorin were associated with ESCC progression and good prognosis. Our results indicate that the positive expressions of FHL1 and Decorin were independent prognostic factors for patients with esophageal cancer, which might be potential valuable biomarkers for ESCC.

Keywords: Decorin, FHL1, prognosis, esophageal squamous cell carcinoma, cancer biomarker

Introduction

Esophageal squamous cell carcinoma (ESCC) is one of the most frequently-occurring human cancers worldwide, and it has caused considerable loss of health and medical resources. Moreover, ESCC is more likely to attack East Asians (especially Chinese people and Japanese people) than Westerners. The number of ESCCs in China occupies almost five in ten of ESCCs around the world [1]. As reported, smoking, impoverished status and red meat consumption are associated with higher risk of ESCC [2]. Though various treatments are carried out to handle this disease, including surgical treatment, chemotherapy, radiotherapy and other supportive treatments, the long-term survival of ESCC patients has not been significantly improved [2]. Hitherto, various molecular biomarker experiments have been conducted to find out some reliable biomarkers that could predict the progression and prognosis of ESCC.

As reported, abnormal expressions of some biomarkers are correlated with the poor prognosis of ESCC, such as positive expression of p53 [3], deregulation of microRNA-335 [4] and overexpression of c-Met [5].

Four and a half LIM domains 1 (FHL1) belongs to the FHL family (including FHL1 to 5) and consists of four and a half LIM domains. Proteins in the FHL family contain only LIM domains which consist of almost 50 amino acid residues and are rich in cysteine with two tandem zinc-finger structures. Initially, FHL1 was only reportedly correlated with cardiac hypertrophy, which can promote the differentiation of myoblasts [6]. However, recent studies imply that FHL1 might serve as a tumor inhibitor and plays an important role in tumorigenesis and progression. As reported, expressions of FHL1 mRNA and protein in gastric carcinoma tissues are significantly lower than in adjacent normal tissues [7]. Lower FHL1 expression is significantly correlat-
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ed with lower degree of differentiation, higher tumor node metastasis (TNM) stage, and greater invasive potential of gastric cancer. Similarly, Decorin has been verified to be another tumor-inhibitor that is correlated with tumorigenesis and progression. Decorin belongs to the extracellular matrix (ECM) small leucine-rich proteoglycan (SLRP) family. As reported, Decorin was deregulated in non-small cell lung cancer (NSCLC) tissues compared with the adjacent normal lung tissues or normal tissues [8]. The deregulation of Decorin is associated with various clinicopathological factors, including tumor size, lymph node metastasis, tumor stage, and prognosis. Overexpression of Decorin could inhibit the proliferation and metastasis of the A549 cell line.

However, there is no clinical experiment exploring the roles of FHL1 and Decorin in ESCC. In this study, we will investigate the expression states of FHL1 and Decorin in ESCC patients, and retrospectively analyze their prognostic values for the first time.

**Material and methods**

**Patients and specimens**

Only patients with complete clinical information were recruited in our experiment. Finally, a total of 82 ESCC tumor tissues and matched adjacent normal esophageal tissues were enrolled from patients at the Fifth People’s Hospital of Shanghai, Fudan University between 2002 and 2008. These patients (64 men and 18 women) were aged 43 to 83 years old (median age 65) and followed up for 10 to 114 months (median 46 months). All specimens were fixed in 10% buffered formalin, embedded in paraffin and stored in the Pathologic Department of our hospital. The clinical stage of each patient was classified or reclassified according to the seventh edition of the American Joint Committee
on Cancer staging system. The overall survival (OS) was defined as the period from diagnosis to ESCC-caused death. This study was approved by the Independent Ethics Committee of our Hospital and all patients signed an informed consent form. All clinical data of patients were collected from hospitalization and subsequent records.

**Immunohistochemical (IHC) staining**

The specimens were made into 4-μm continuous sections for IHC analysis. IHC evaluation was performed using a rabbit anti-human FHL1 polyclonal primary antibody (Abcam, ab49241) and a rabbit anti-human Decorin polyclonal primary (Abcam, ab67449). The IHC staining was carried out according to the protocol of a detection kit (Shanghai BioSun Sci&Tech Co., Ltd). The staining intensities of FHL1 and Decorin were evaluated by two pathologists who were blind to the diagnosis of individual patients. The score assessment was based on addition between staining intensity and percentage of positive cells. In particular, staining intensity is scored according to a three-tier system: 0, no staining; 1, weak; 2, moderate; 3, strong. The percentage of positive cells was scored based on a four-tier system: 0, 0%; 1, ≤ 15%; 2, ≤ 30%; 3, ≤ 45%; 4, > 45%. Finally, the negative and positive expressions are defined as total score ≤ 2 and ≥ 3 respectively [9].

**Statistical analysis**

All analyses were performed using SPSS 19.0 (IBM, Armonk, NY). Various clinicopathologic factors in different FHL1 or Decorin expression groups were examined, including: sex, age, tumor differentiation, metastasis, T stage and clinical stage. Categorical variables were compared with the pared Chi-square test. Survival curves were plotted using the Kaplan-Meier method and compared using the log-rank test. Multivariate survival analysis was performed to identify independent prognostic factor, using the Cox regression model for OS. P < 0.05 was considered statistically significant.

**Results**

**Deregulation of FHL1 and decorin in ESCC**

FHL1 and Decorin are both localized primarily in the cytoplasm (Figure 1). The expression levels of FHL1 and Decorin in ESCCs are 42.7% and 34.1%, respectively, but are 93.9% and 87.8%, respectively in normal tissues. We find that FHL1 and Decorin are significantly deregulated in ESCC, compared with normal tissues (P < 0.05).

**Relationship between clinicopathologic factors and FHL1 or decorin expression in ESCC**

The results show that positive expression of FHL1 is significantly associated with differentiation (P = 0.008), metastasis (P = 0.000), T stage (P = 0.000) and clinical stage (P = 0.000). Also positive expression of Decorin is significantly associated with metastasis (P = 0.001), T stage (P = 0.001) and clinical stage (P = 0.000). In addition, the relationships between clinicopathologic factors and FHL1 or Decorin expression are elaborated in Table 1.
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Prognostic role of expression status of FHL1 and decorin in ESCC

The Kaplan-Meier analysis shows that patients with negative FHL1 expression in ESCC have a significantly shorter OS period than those patients with positive FHL1 expression (median survival time 35 vs. 61 months; P < 0.001) ([Figure 2A](#)). Similarly, the negative expression of Decorin in ESCC is significantly correlated with poor prognosis (P < 0.05) ([Figure 2B](#)). The median survival time of Decorin-negative and -positive patients is 37 and 56 months, respectively. In addition, patients with positive expression of both FHL1 and Decorin have a significantly better prognosis than patients with negative expression of both FHL1 and Decorin (P < 0.001) ([Figure 3](#)). Multivariable analysis indicates that positive expressions of FHL1 (HR = 0.341, 95% CI: 0.118-0.986, P = 0.047) and Decorin (HR = 0.448, 95% CI: 0.216-0.930, P = 0.031) are both significant independent predictors of poor prognosis ([Table 2](#)).

Discussion

So far as we know, this is the first study explaining the prognostic role of deregulation of FHL1 and Decorin in ESCC patients. We find that both molecular biomarkers are significantly deregulated in ESCC tissues, compared with adjacent normal esophageal tissues. Furthermore, the loss of the two biomarkers is significantly correlated with several clinicopathological factors, such as differentiation, lymph node metastasis, T stage and clinical stage. Homogeneously, the loss of the two biomarkers has also been reported. For example, the expression of FHL1 in lung cancer tissues was decreased, compared to non-cancerous tissues [8]. FHL1 expression in tumor tissues is frequently down-regulated compared with normal mucosa [10]. As reported, various human cancers are found with deregulation of Decorin, including hepatocellular carcinoma [11], oral cancer [12], breast carcinoma [13], prostate tumor [14], and colorectal cancer [15].

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**Figure 2.** Overall survival (OS) curves. A. OS curves of patients in relation to FHL1 expression; B. OS curves of patients in relation to Decorin expression.
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We also analyzed the prognostic role of deregulation of FHL1 and/or Decorin in ESCC patients. Our results indicate that patients with negative FHL1 expression have a significantly shorter OS than those with positive FHL1 expression. Our results coincide with another study [10] that patients with low FHL1 expression tumor show significantly shorter survival than those with high FHL1 expression tumor. Similarly, the deregulation of Decorin in ESCC patients predicts a poor prognosis. Multivariate survival analysis indicates that deregulations of FHL1 and Decorin are both independent indicators for poor prognosis of ESCC.

For tumor-inhibiting factors, we have no enough knowledge about how the deregulation of FHL1 and Decorin is associated with the prognosis and progression of cancers. As reported, FHL1 impacts the progression of cancers by regulating the cycle of tumor cells. Cell line and animal experiments show that FHL1 increases the expression of p21 (a CDK inhibitor) through regulation of gene transcription in lung cancer cells [10]. Also p21 plays a role in the maintenance of G2/M-phase arrest, and the regulation of cell cycle arrest at the G1/S transition. FHL1 induces the upregulation of p21 and inhibits cyclins and their associated CDKs (mainly cyclin D1), which are the central machinery governing cell cycle progression. Finally, deregulation of FHL1 promotes the growth of anchorage-dependent and -independent lung cancer cells. In breast cancers, FHL1 could interact with the estrogen receptor and regulate the growth of breast cancer cells [16].

Decorin is a member of SLRP family. It is demonstrated that abnormal expression of Decorin might impact tumor progression through several signal pathways [17]. Firstly, Decorin competes with EGF for a receptor binding site on the surface of tumor cells, and then inhibits tumor cell proliferation. Decorin also can suppress the activity of the ErbB2 and ErbB4 receptors, interact with Met, and inhibit cell migration and growth [18]. Secondly, Decorin might play a role in tumor angiogenesis through binding to the transforming growth factor (TGF)-β and interacting with the TGF-β signal pathway [19]. Thirdly, Decorin might be associated with the loss of E-cadherin, and plays a significant role in tumor metastasis [17].

In conclusion, this is the first study focusing on evaluating the prognostic roles of FHL1 and Decorin expressions in ESCC patients. The results show that FHL1 and Decorin positive

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**Table 2.** Multivariable analysis of the prognostic roles for clinopathological factors in patients with esophageal squamous cell carcinoma (ESCC)

<table>
<thead>
<tr>
<th></th>
<th>HR</th>
<th>95.0% CI</th>
<th>P</th>
</tr>
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<tr>
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<td>0.584</td>
<td>0.316</td>
<td>0.782</td>
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<tr>
<td>T stage</td>
<td>0.728</td>
<td>0.624</td>
<td>0.847</td>
</tr>
<tr>
<td>Differentiation</td>
<td>0.684</td>
<td>0.314</td>
<td>0.872</td>
</tr>
<tr>
<td>Lymph node metastasis</td>
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<td>0.504</td>
<td>0.946</td>
</tr>
<tr>
<td>FHL1 positive</td>
<td>0.341</td>
<td>0.118</td>
<td>0.986</td>
</tr>
<tr>
<td>Decorin positive</td>
<td>0.448</td>
<td>0.216</td>
<td>0.930</td>
</tr>
</tbody>
</table>

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**Figure 3.** Overall survival (OS) curves using combinations of FHL1 and Decorin expression statues.
expressions are associated with favorable prognosis in ESCC patients. Furthermore, FHL1 and Decorin are both independent predictors of better overall survival in ESCC patients. In the future, we aim to get a more precise evaluation on the prognostic role of FHL1 and Decorin deregulation in ESCC patients, and thus more studies with large sample size and long-time follow-up are needed.

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Disclosure of conflicts of interest

None.

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