

# Expression of hypoxia-inducible factor (HIF)-1 $\alpha$ and vascular endothelial growth factor (VEGF)-D as outcome predictors in resected esophageal squamous cell carcinoma

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**Abstract.** Hypoxia-inducible factor (HIF)-1 $\alpha$  and vascular endothelial growth factor (VEGF) are important angiogenic factors in human cancers. Relative to VEGF-C, prognostic significance of VEGF-D expression and its association with HIF-1 $\alpha$  expression remain elusive in esophageal squamous cell cancer (ESCC). We studied expression of HIF-1 $\alpha$  and VEGF-D using immunohistochemistry in 85 resected ESCC specimens and correlated results with patients' clinicopathologic parameters and survival. Association between expression of HIF-1 $\alpha$  and VEGF-D was investigated using a concordance analysis. High expression of HIF-1 $\alpha$  and VEGF-D was observed in 52 (61.2%) and 56 (65.9%) patients, respectively. HIF-1 $\alpha$  expression correlated well with tumor stage ( $P = 0.041$ ), whereas VEGF-D expression correlated with tumor stage ( $P = 0.027$ ) and N status ( $P = 0.019$ ). Groups of high HIF-1 $\alpha$  and VEGF-D showed worse survivals than those of low expression ( $P = 0.002$  and  $0.001$ , respectively). Multivariate analysis supported expression of HIF-1 $\alpha$  and VEGF-D as significant survival predictors ( $P = 0.044$  and  $0.035$ , respectively). A concordance rate of 69.5% was observed between expression of HIF-1 $\alpha$  and VEGF-D. In conclusion, protein expression of HIF-1 $\alpha$  and VEGF-D are independent prognostic predictors. An association between expression of HIF-1 $\alpha$  and VEGF-D suggests that these two angiogenic factors are essential in progression of ESCC.

**Keywords:** Hypoxia-inducible Factor (HIF)-1 $\alpha$ , vascular endothelial growth factor (VEGF)-D, esophageal squamous cell carcinoma, prognosis, survival

## 1. Introduction

Angiogenesis is essential for tumor growth and its spread, including invasion and metastasis [8]. This

process relies on production of a number of angiogenic factors by tumor and surrounding host cells [9]. A key regulator in this process is the transcription factor hypoxia-inducible factor-1 (HIF-1), which targets several genes related to cell proliferation, angiogenesis or invasion in response to a hypoxic microenvironment within the tumor [18,21,34].

HIF-1 is a heterodimeric basic helix–loop–helix protein composed of HIF-1 $\alpha$  and HIF-1 $\beta$  subunits [24]. Under normoxic conditions, the HIF-1 $\alpha$  subunit is subjected to ubiquitination and proteosomal degradation;

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whereas in hypoxia, its degradation is inhibited, and its expression increases rapidly [42]. Tumors derived from cells lacking HIF-1 $\alpha$  or HIF-1 $\beta$  show significantly reduced vascularization and often reduced growth rates compared with parental cells [4,32].

A key angiogenic factor whose gene expression is regulated by HIF-1 is the vascular endothelial growth factor (VEGF) [21]. Under hypoxic conditions within the tumor cells, HIF-1 $\alpha$  binds to a DNA recognition sequence in the promoter of target genes, including the VEGF gene [30]. Thus, under hypoxic conditions, VEGF transcription is up-regulated by HIF, and additionally hypoxia promotes the stabilization of VEGF mRNA by proteins that bind to sequences in the 3' untranslated region (3' UTR) of the mRNA [6].

VEGF is a family of the platelet-derived growth factor composed of 6 VEGF members including VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, and placenta growth factor (PlGF) [41]. The prognostic significance of VEGF expression has been reported in gastric, colon, breast, bladder and esophageal carcinomas, as well as in other malignancies [7,16,17,31]. Notably, previous studies for gastrointestinal malignancies have focused on expression of VEGF-C in relation to lymph node metastasis and survival, including gastric, esophageal, and colorectal carcinomas [2,10,22]. It remains controversial whether VEGF-D promotes angiogenesis and lymphangiogenesis like VEGF-C in human cancers [26,29,37]. In view of lack of correlative study of VEGF-D expression with clinical outcome in patients with esophageal squamous cell carcinoma (ESCC), this study aimed to clarify prognostic significance of expression of VEGF-D and HIF-1 $\alpha$  in the same series, and to investigate whether there is an association between expression of HIF-1 $\alpha$  and VEGF-D in resected ESCC.

## 2. Research design and methods

### 2.1. Study population and tumor samples

Archival paraffin-embedded tissue blocks were obtained retrospectively of primary tumor specimens from 85 patients who underwent esophagectomy from January 1997 to December 2001. These were serial patients who had a complete resection of the tumor and availability of sufficient follow-up data with an exclusion of in-hospital mortality. Patients included in this study did not receive preoperative chemotherapy or radiotherapy. Waiver of patient consent was granted by

the Institutional Review Board, which allowed us to get access to patients' medical records and to obtain tissue samples and pertinent follow-up data. From review of medical records, clinical information was collected and TNM status was recorded based upon the American Joint Committee on Cancer (AJCC) staging system [12] for esophageal cancer. Survival and follow-up data were obtained from the Institutional Cancer Registry.

### 2.2. Analysis of protein expression by immunohistochemistry (IHC)

Paraffin blocks of tumors were cut into 5  $\mu$ m slices and then processed using standard deparaffinization and rehydration techniques. After antigen retrieval using microwave heating, all tissue sections on slides were immunostained following instructions from the antibody suppliers. A monoclonal anti-HIF-1 $\alpha$  antibody clone H1alpha67 (1:200; Novus Biologicals, Inc., Littleton, CO, USA) was used to detect HIF-1 $\alpha$  protein and a monoclonal antibody clone 78923.11 against human VEGF-D (1:100; R&D Systems, Minneapolis, MN, USA) was used to detect VEGF-D, respectively. The binding of primary antibody was visualized using a detection system (DAKO LSAB Kit K675; DakoCytomation California Inc., Carpinteria, California, USA). The normal staining patterns for HIF-1 $\alpha$  are nuclear and/or cytoplasmic, whereas that for VEGF-D is cytoplasmic. Tumor cells that exhibited an absence of staining in the presence of surrounding non-neoplastic cells with cytoplasmic or nuclear staining were considered to have an abnormal pattern. Staining results were examined by two board-certified pathologists masked to patients' clinical information. Another reading by a third observer was needed to reach a consensus when there was a significant discrepancy between initial readings. The percentage of the tumor cells that exhibited a positive immunoreactivity was determined at x 200 magnification using a light microscope (Olympus BX51, Olympus America Inc.). To be in consensus with most studies of expression HIF-1 $\alpha$  and VEGF in tumors by immunohistochemistry, we used scoring systems to distinguish low from high expression of HIF-1 $\alpha$  [1,19] and VEGF-D [20,36] as previously described respectively. Briefly, both the percentage of positive cells and the intensity of the reaction were taken into account; 0, no staining; 1, <1% positive cells; 2, 1–10% positive cells with slight to moderate reaction; 3, 10–50% positive cells with moderate to high reaction and 4, >50% positive cells with

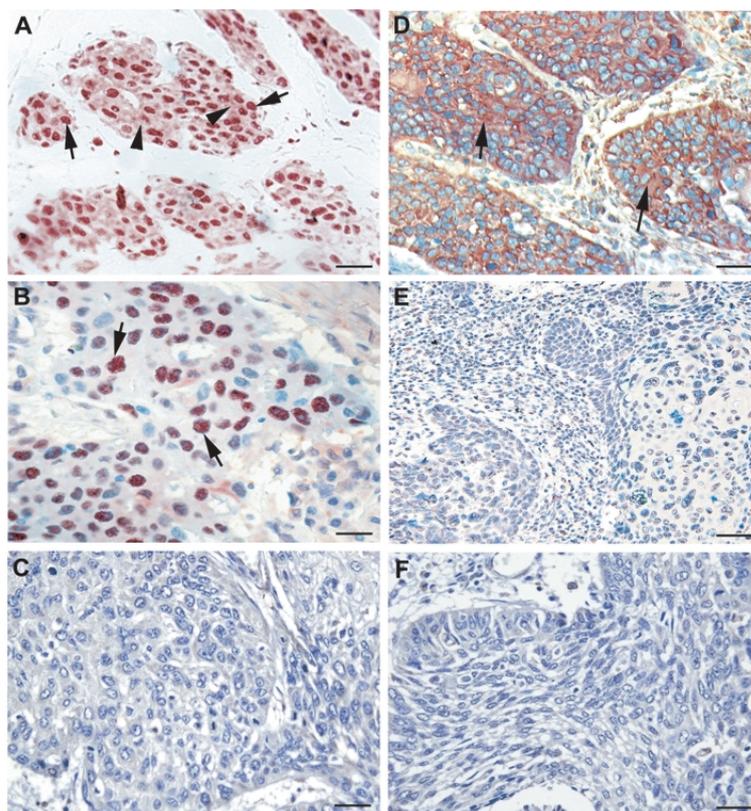


Fig. 1. Representative results of immunostaining for HIF-1 $\alpha$  (panels A–C) and VEGF-D (panels D–F) in resected specimens of esophageal squamous cell carcinoma. A. Positive HIF-1 $\alpha$  immunoreactivity was detected in nuclei (arrows) and cytoplasm (arrowheads) of tumor cells. B. Positive HIF-1 $\alpha$  immunoreactivity was detected only in nuclei of tumor cells. D. Positive immunoreactivity for VEGF-D was detected in the cytoplasm of tumor cells (arrows). E. Tumor cells stained negatively (arrows) for VEGF-D. C and F. Negative controls for immunostaining of HIF-1 $\alpha$  and VEGF-D stained negatively for all cells. Bars: A, C, D and F = 50  $\mu$ m; B = 40  $\mu$ m; E = 100  $\mu$ m.

moderate to high reaction. Staining scored as 0, 1, and 2 was considered as low expression, whereas the rest were considered as high expression. Mouse isotype-matched IgG2b and IgG<sub>1</sub> served as negative controls for HIF-1 $\alpha$  and VEGF-D antibody, respectively.

### 2.3. Statistical analysis

The Pearson $\chi^2$  or Fisher exact test as appropriate was used to compare expression of HIF-1 $\alpha$  and VEGF-D protein with different clinicopathologic variables of the patients, including age, sex, tumor stage, and TNM category. The Kaplan-Meier method [14] was used to estimate the probability of survival as a function of time and median survival following esophagectomy. The log-rank test was used to assess the significance of difference between pairs of survival probabilities [39]. Multivariate analysis for survival was performed using the Cox proportional hazards models. Statistical analysis was performed using the SPSS software package,

version 13.0 (SPSS Inc, Chicago, IL). A *P* value less than 0.05 was considered statistically significant.

## 3. Results

### 3.1. Protein expression of HIF-1 $\alpha$ and VEGF-D in relation to patients' clinicopathologic variables

Representative immunohistochemistry results for protein expression of HIF-1 $\alpha$  and VEGF-D are shown in Fig. 1. The comparisons of protein expression of HIF-1 $\alpha$  and VEGF-D in relation to patients' clinicopathologic variables were summarized in Table 1. High expression of HIF-1 $\alpha$  and VEGF-D was observed in 52 (61.2%) and 56 (65.9%) patients, respectively. Expression of HIF-1 $\alpha$  correlated positively with tumor stage only (*P* = 0.041), whereas expression of VEGF-D showed significant positive correlation with tumor stage (*P* = 0.027) and N (nodal metastasis) (*P* = 0.019).

Table 1  
Comparison between protein expression of HIF-1 $\alpha$  and VEGF-D, with clinicopathologic features for patients undergoing esophagectomy for squamous cell carcinoma of the esophagus

| Characteristics | Patients | HIF-1 $\alpha$ expression |                 | VEGF-D expression |                 |
|-----------------|----------|---------------------------|-----------------|-------------------|-----------------|
|                 |          | High                      | Low (%)         | High              | Low (%)         |
| Overall         | 85       | 52 (61.2%)                | 33 (38.8%)      | 56 (65.9%)        | 29 (34.1%)      |
| Age             |          |                           |                 |                   |                 |
| $\leq 70$       | 60       | 36                        | 24              | 37                | 23              |
| $> 70$          | 25       | 16                        | 9               | 19                | 6               |
| Sex             |          |                           |                 |                   |                 |
| M               | 80       | 47                        | 33              | 52                | 28              |
| F               | 5        | 5                         | 0               | 4                 | 1               |
| Staging         |          |                           |                 |                   |                 |
| I+II            | 48       | 25                        | 23 <sup>a</sup> | 27                | 21 <sup>b</sup> |
| III+IV          | 37       | 27                        | 10              | 29                | 8               |
| TNM             |          |                           |                 |                   |                 |
| T1+2            | 30       | 16                        | 14              | 17                | 13              |
| T3+4            | 55       | 36                        | 19              | 39                | 16              |
| N0              | 44       | 23                        | 21              | 24                | 20 <sup>c</sup> |
| N1              | 41       | 29                        | 12              | 32                | 9               |
| M0              | 68       | 39                        | 29              | 44                | 24              |
| M1              | 17       | 13                        | 4               | 12                | 5               |

<sup>a,b</sup> $P = 0.041$  and  $0.027$  between stage I+II and III+IV, respectively; <sup>c</sup> $P = 0.019$  between N0 and N1.

TNM: T, tumor invasion; N, regional lymph nodes; M, distant sites (metastases).

Table 2  
Univariate Kaplan-Meier analysis for clinicopathologic variables and expression of HIF-1 $\alpha$  and VEGF-D

| Variables        | Categories | N  | Survival time (months)<br>Median $\pm$ SE (95% C.I.) | $P$ value   |
|------------------|------------|----|--|-------------|
| Age (years)      | $\leq 70$  | 55 | 19.0 $\pm$ 8.5 (2.4–35.6)                            | 0.675       |
|                  | $> 70$     | 30 | 18.0 $\pm$ 6.2 (5.9–30.1)                            |             |
| Sex              | Male       | 80 | 19.0 $\pm$ 5.0 (9.1–28.8)                            | 0.222       |
|                  | Female     | 5  | 15.0 $\pm$ 3.3 (8.5–21.4)                            |             |
| Tumor status (T) | T1         | 7  | 29.0 $\pm$ 20.9 (0.0–70.1)                           | 0.032*      |
|                  | T2         | 23 | 29.0 $\pm$ 7.2 (14.9–43.1)                           |             |
|                  | T3         | 48 | 15.0 $\pm$ 6.9 (1.4–28.6)                            |             |
|                  | T4         | 7  | 9.0 $\pm$ 2.6 (3.9–14.1)                             |             |
| Nodal status (N) | N0         | 44 | 29.0 $\pm$ 0.9 (27.1–30.9)                           | $< 0.001^*$ |
|                  | N1         | 41 | 15.0 $\pm$ 3.9 (7.4–22.7)                            |             |
| Metastasis (M)   | M0         | 68 | 22.0 $\pm$ 5.7 (10.9–33.1)                           | 0.007*      |
|                  | M1         | 17 | 10.0 $\pm$ 3.4 (3.3–16.6)                            |             |
| Stage            | I          | 5  | 30.0 $\pm$ 0.4 (29.1–30.9.2)                         | $< 0.001^*$ |
|                  | II         | 43 | 29.0 $\pm$ 2.6 (23.9–34.1)                           |             |
|                  | III        | 22 | 10.0 $\pm$ 0.9 (8.2–11.8)                            |             |
|                  | IV         | 15 | 6.0 $\pm$ 2.9 (0.3–11.7)                             |             |
| HIF-1 $\alpha$   | Low        | 33 | 30.0 $\pm$ 1.1 (27.8–32.2)                           | 0.002*      |
|                  | High       | 52 | 12.0 $\pm$ 2.4 (7.3–16.7)                            |             |
| VEGF-D           | Low        | 29 | 33.0 $\pm$ 3.6 (25.9–40.0)                           | 0.001*      |
|                  | High       | 56 | 12.0 $\pm$ 2.3 (7.4–16.6)                            |             |

\*Difference is considered significant when  $P < 0.05$ .

### 3.2. Survival analysis in relation to clinicopathologic variables including protein expression of HIF-1 $\alpha$ and VEGF-D

Univariate Kaplan-Meier analysis for clinicopathologic variables and expression of HIF-1 $\alpha$  and VEGF-

D was performed to determine survival predictor(s) and the results were summarized in Table 2. Among all variables analyzed, the groups of high expression of HIF-1 $\alpha$  and VEGF-D showed worse survivals than those of low expression ( $P = 0.002$ ) and ( $P = 0.001$ ), respectively (Fig. 2). Median survival durations in

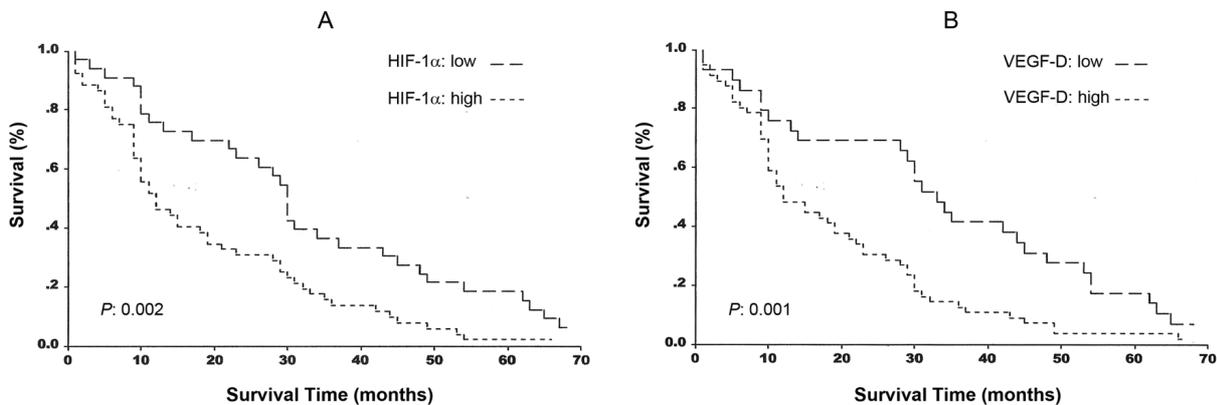


Fig. 2. Kaplan-Meier survival curve with log-rank test for 85 patients after resection for esophageal squamous cell carcinoma. Comparison of survival between patients with low and high expression of HIF-1 $\alpha$  protein and VEGF-D is shown in (A) and (B), respectively. Ninety five percent of confidence intervals (C.I.) for median survival in groups of low and high expression of HIF-1 $\alpha$  were 27.8–32.2 and 7.3–16.7, and in low and high VEGF-D groups, were 25.9–40.0 and 7.4–16.6, respectively.

months were  $30.0 \pm 1.1$  and  $12.0 \pm 2.4$  (SE) for patients with low and high expression of HIF-1 $\alpha$ , and  $33.0 \pm 3.6$  and  $12.0 \pm 2.3$  for patients with low and high VEGF-D expression, respectively. Multivariate analysis for prediction of survival showed that tumor stage, expression of HIF-1 $\alpha$  and VEGF-D were independent survival predictors with a  $P = 0.019$ , 0.044 and 0.035, respectively (Table 3).

### 3.3. Correlation of protein expression of HIF-1 $\alpha$ with protein expression of VEGF-D

Results of high HIF-1 $\alpha$ /VEGF-D and low HIF-1 $\alpha$ /VEGF-D expressions were considered concordant. A concordance rate of 69.5% was observed between protein expression of HIF-1 $\alpha$  and VEGF-D ( $P = 0.002$ ) (Table 4).

## 4. Discussion

Previous studies on tumor angiogenesis have suggested an important role of HIF-1 as an inducer for angiogenic factors in hypoxic tumor microenvironment [8,9,18,34]. Among genes whose expression is regulated by HIF-1, VEGF is well-documented as a key angiogenic factor [21]. Correlation of expression of these two factors with clinical outcome has been widely investigated in a variety of human cancers including esophageal cancer [16,17,22]. Most of these studies on esophageal cancer have focused on expression of VEGF-C [17,22]. In view of lack of investigation concerning VEGF-D expression and association study for

Table 3  
Multivariate analysis using a Cox proportional hazards model for variables including patients' clinicopathologic features and expression of HIF-1 $\alpha$  and VEGF-D

| Variable       | HR    | 95% CI       | P      |
|----------------|-------|--------------|--------|
| Age            | 1.014 | 0.992–1.037  | 0.214  |
| Sex            | 0.729 | 0.275–1.938  | 0.527  |
| Stage          | 3.884 | 1.244–12.125 | 0.019* |
| T status       | 0.793 | 0.537–1.170  | 0.242  |
| N status       | 1.272 | 0.474–3.413  | 0.632  |
| M status       | 1.158 | 0.570–2.350  | 0.685  |
| HIF-1 $\alpha$ | 1.768 | 1.053–2.970  | 0.044* |
| VEGF-D         | 1.738 | 1.035–2.920  | 0.035* |

\*Difference is considered significant when  $P < 0.05$ .

Table 4  
Concordance analysis between protein expression of HIF-1 $\alpha$  and VEGF-D in resected esophageal squamous cell carcinoma

| VEGF-D                   | HIF-1 $\alpha$          |                         |
|--------------------------|-------------------------|-------------------------|
|                          | High                    | Low                     |
| High                     | 41 (48.3%)*             | 11 (12.9%) <sup>†</sup> |
| Low                      | 15 (17.6%) <sup>†</sup> | 18 (21.2%)*             |
| Concordant vs discordant | 59 (69.5%)*             | 26 (30.5%) <sup>†</sup> |

Data were presented as actual numbers and percentages of cases in a total of 85 patients. \*Concordant: high HIF-1 $\alpha$ /VEGF-D and low HIF-1 $\alpha$ /VEGF-D expressions. <sup>†</sup>Discordant: high HIF-1 $\alpha$ /low VEGF-D and low HIF-1 $\alpha$ /high VEGF-D expressions.  $P = 0.002$  for correlation between protein expression of HIF-1 $\alpha$  and VEGF-D.

expression of HIF-1 $\alpha$  and VEGF-D in esophageal squamous cell carcinoma, we designed this study to investigate prognostic significance of expression of VEGF-D and HIF-1 $\alpha$  and the relationship between expressions of these two factors in a cohort of 85 resected ESCC.

Expression of HIF-1 $\alpha$  is enhanced by genetic alterations in tumor suppressor genes (*von Hippel-Lindau/VHL*, *p53*, *PTEN*) and oncogenes (*v-src*,

*HER2neu*, *H-ras*) and by the induction of several growth factors (insulin-like growth factor (IGF)-1 and IGF-2, basic fibroblast growth factor (bFGF), and epidermal growth factor (EGF) [16,21]. HIF-1 $\alpha$  activates transcription of VEGF through a direct binding HIF-1 $\alpha$  binding site [6,13]. Inhibition of HIF-1 $\alpha$  results in down-regulation of VEGF and increased apoptosis of cancer cells [23,44], indicating that HIF-1 $\alpha$  may serve as a potential target for cancer therapy while supporting its role as a key factor in tumor angiogenesis and tumor progression. HIF-1 $\alpha$  is absent in most normal tissues and its IHC staining patterns vary depending on different tissue types [45]. Consistent with staining pattern for HIF-1 $\alpha$  in ESCC [16], our results of HIF-1 $\alpha$  immunostaining also showed nuclear and/or cytoplasmic immunoreactivity in tumor cells.

Recent studies have shown that HIF-1 $\alpha$  expression correlates with VEGF expression and high expression of these two factors are generally considered as poor prognostic predictors in a various human cancers including esophageal cancer [1,3,16]. Kimura and associates reported a 39% of high expression of HIF-1 $\alpha$  in 32 out of their 82 ESCC tumor specimens and concluded that higher expression of HIF-1 $\alpha$  correlated positively with vascular invasion and microvessel density within the tumor but not tumor stage, individual TNM factors or survival for the entire patient cohort. In contrast, our results showed a higher rate of high HIF-1 $\alpha$  expression, 52 in 85 (61.2%), of resected tumor specimens. Unlike their results, the expression of HIF-1 $\alpha$  correlated positively with tumor stage and inversely with patients' survival in our study. Discrepancies from these two studies may arise from different populations studied; for instance, different ethnic groups or their inclusion of patients with preoperative induction therapy. One may speculate that pre-operative induction therapy may alter HIF-1 $\alpha$  expression in ESCC. Similar studies on head neck cancer did not show prognostic impact on patients' survival in relation to HIF-1 $\alpha$  expression [1]. For breast cancer, Dales and associates concluded that high HIF-1 $\alpha$  expression correlated with high risk of metastasis and poor survival [5]. Taken together, these results suggest that prognostic significance of expression of HIF-1 $\alpha$  may vary among different cancer types.

Following induction by HIF-1, VEGF is produced in concert with a number of growth factors such as EGF, bFGF, platelet-derived growth factor (PDGF), and transforming growth factor (TGF)- $\beta$  to promote tumor angiogenesis [16,40]. These stimulatory factors for VEGF are considered to be produced, in an autocrine and paracrine fashion, from tumor cells, stromal

cells, or both by the interaction of tumor cells and host cells [27]. Employing an animal model, VEGF-C and VEGF-D have been shown to induce lymphangiogenesis that is associated with lymph node metastasis [15, 37]. Previous studies on the role of VEGF in tumor angiogenesis and its prognostic significance in different type of human cancers mostly focused on VEGF-C [7, 16,17,31,43], leaving limited reports on VEGF-D. Increased VEGF-D expression is associated with lymph node metastasis in colon and prostate [29,43] cancer but not in gastric cancer [35]. In contrast, a few reports demonstrate that expression of VEGF-D is down-regulated in tumor tissues compared to that in adjacent normal tissues [11,25], suggesting that VEGF-D may have anti-angiogenic effect relative to VEGF-C. In current study, expression of VEGF-D in ESCC correlated positively with tumor stage and nodal metastasis. Further, we have demonstrated that along with HIF-1 $\alpha$ , VEGF-D serves as an independent survival predictor for patients undergoing esophagectomy for ESCC. These results were in general agreement with results from a study that compared prognostic significance VEGF-C and VEGF-D expression in patients with colorectal cancer, showing that both of these two factors correlated positively with tumor invasion and nodal metastasis but inversely with overall survival [29]. In contrast, Shida and associates compared expression of VEGF-C and VEGF-D in gastric cancer and demonstrated that only VEGF-C correlated with patients' clinicopathologic variables [35]. These results suggest that prognostic impact of VEGF-D expression may vary in different types of human cancers.

Previous studies rarely investigated relationship between expression of HIF-1 $\alpha$  and VEGF-D in the same series of cancer study. A selective report by Kimura and associates demonstrated that expression HIF-1 $\alpha$  is associated with that of VEGF-C in ESCC and both of these two factors predict patients' survival [16]. In comparison, we have demonstrated that expression of VEGF-D, relative to VEGF-C, was also associated with expression of HIF-1 $\alpha$  in resected ESCC, for which supported by a significant concordance (69.5%) between expression of HIF-1 $\alpha$  and VEGF-D. Taken together, the results may imply that different types of VEGF may function synergistically with HIF-1 $\alpha$  in promoting tumor angiogenesis. This speculation needs further investigation on their gene expression and biological functions in relation to tumor angiogenesis using *in vitro* and/or *in vivo* models.

The discovery of HIF-1 makes it a potential molecular target for the development of cancer therapeutics.

The identification of selective HIF-1inhibitors would not only be useful for the potential therapeutic implications but also for their application as analytic tools to further define the role of HIF in human cancers. Continued efforts have been made in the development of HIF inhibitors, such as synthetic polyamides that inhibit HIF-1 DNA binding [28] and more recently identified small molecule inhibitors (e.g. 103D5R) that selectively blocks HIF-1 protein synthesis [38]. On the other hand, bevacizumab, an anti-VEGF monoclonal antibody, has been proven to be an effective target therapy in a variety of cancers [33]. Our results support a role of HIF-1 and VEGF-D as predictors for poor prognosis in ESCC and provide a reasonable rationale for further investigation *in vitro* and *in vivo* with the effects of HIF-1 inhibitors and VEGF-D-targeted therapy for ESCC.

In conclusion, results from this study supported a role of expression of HIF-1 $\alpha$  and VEGF-D as independent survival predictors in resected ESCC and suggested that they may serve as potential therapeutic targets for ESCC. A good correlation between expression of HIF-1 $\alpha$  and VEGF-D suggests that both of these two angiogenic factors are essential for tumor progression or angiogenesis of ESCC.

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