Evaluation of serum HGF and CK18 levels in patients with esophageal cancer

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ABSTRACT. Cytokeratins are thought to play a role in apoptosis. Cytokeratin 18 (CK18) is involved in the formation of intracellular cytoskeleton, and has been considered a promising apoptosis marker in gastrointestinal carcinomas. Growth factors, including hepatocyte growth factor (HGF), may provide a microenvironment for malignant cells. In this study, we aimed to compare serum HGF and CK18 levels between esophageal squamous cell carcinoma patients and healthy controls. The study included 41 adult patients (20 male, 21 female) diagnosed with esophageal squamous cell carcinoma, with a mean age of 63.54 ± 10.88 years (range, 41-82 years). We also recruited 39 age

and gender-matched healthy control subjects. Venous blood samples were taken; serum HGF and CK18 concentrations were determined via ELISA. Results indicated that serum HGF levels were higher in patients (1.37 ± 0.63 ng/mL) as compared to the healthy subjects (0.41 ± 0.29 ng/mL). Similarly, serum CK18 levels were higher in the patient group (2.53 ± 1.33 ng/mL) than in the control group (0.34 ± 0.23 ng/mL) ($P < 0.001$). In addition, serum HGF and CK18 levels were positively correlated with metastasis stage, tumor stage, and disease stage of esophageal squamous cell carcinoma. To our knowledge, this is the first study to evaluate serum HGF and CK18 levels in patients with esophageal squamous cell carcinoma. The results suggest that serum CK18 and HGF levels may be used as prognostic and disease monitoring biomarkers of esophageal squamous cell carcinoma.

Key words: HGF; CK18; Esophageal squamous cell carcinoma; Biomarker

INTRODUCTION

Esophageal squamous cell carcinoma (ESCC) is a sub-type of human epithelial cancer, and is one of the most lethal carcinomas worldwide (Guo et al., 2008). As the disease is usually diagnosed in late stages, it has poor prognosis with high metastasis rates. The 5-year survival rates vary between 15 and 25% (Pennathur et al., 2013). Interactions between genetic and environmental factors are involved in the pathogenesis of this disease (Parker et al., 2010). Surgical operation, preoperative and postoperative adjuvant chemotherapy, as well as preoperative chemoradiotherapy are the most common treatment options for ESCC.

Histopathological examination of resected tissues is the most reliable evidence for the diagnosis of ESCC, yet it is not applicable in non-operated cases. Therefore, the ability to identify biomarkers for early diagnosis of ESCC may improve disease prognosis and survival of ESCC patients.

Cytokeratins (CKs) are a family of intermediate filament proteins that form the intracellular cytoskeleton of epithelial cells (Chu and Weiss, 2002). There are 20 different CK subtypes; CKs with acidic properties are classified as Type I CKs (CK9-20), and those with neutral basic properties are known as Type II CKs (CK1-8). CK18 is a Type I CK, and is a component of the cell's structural network that extends from the surface of the nucleus to the cell membrane. CK18 is involved in important cellular processes including apoptosis, mitosis, cell cycle progression, and cell signaling under normal physiological conditions. Circulating CK18 fragments have been accepted as an epithelial marker for diagnostic histopathological examinations, and has been suggested to affect tumor cell behaviors (Weng et al., 2012).

CK18 is reported to be expressed in various cancerous tissues including ESCC (Kalantari, 2009). Its expression has also been detected in human lung adenocarcinoma cell lines, melanoma, ESCC, as well as some mouse carcinomas (Zhang et al., 2011). CK18 expression patterns have been reported to be associated with lymph node metastasis in ESCC. Furthermore, it has been suggested that CK18 expression and distribution pattern could be used as predictive biomarkers of disease progression (Cintorino et al., 2001).

Human growth factor (HGF) plays a potential role in the progression of various types

of tumors, and is overexpressed in ESCC (Saeki et al., 2002). c-Met is the transmembrane tyrosine kinase receptor of HGF, and possesses similar oncogenic properties. It has been suggested that the HGF/c-Met signaling pathway is associated with malignant cancers, and may be involved with cancer metastasis (Anderson et al., 2006). Conversely, recent studies have shown that HGF may exhibit therapeutic effects on some diseases such as human silicosis (Liu et al., 2015) and myocardial ischemic injury (Wang et al., 2014).

The possible role of HGF and CK18 in the development and progression of ESCC remains unclear. This study aimed to evaluate serum CK18 and HGF levels in ESCC patients, and to investigate the relationship between ESCC and these candidate biomarkers.

MATERIAL AND METHODS

The study included 41 Caucasian patients (female/male: 21/20) diagnosed with ESCC in our clinic as well as 39 healthy subjects (female/male: 20/19).

Subject demographics, as well as clinical and laboratory findings were recorded by the same researcher. Venous blood samples were collected in the morning following an overnight fast. Sera were obtained and stored at -80°C until analysis. Serum CK18 and HGF levels were measured in all subjects using the human Cusabio ELISA kits (CSB-E09172h for CK18 and CSB-E04573h for HGF, Wuhan, China). Intra-assay and interassay coefficient variations of the kit were <8 and <10%, respectively.

ESCC of the thoracic esophagus was confirmed by histopathological examination on biopsy materials obtained via endoscopy. The patients included in the study had no history of other cancers. The clinical distribution of the patients was as follows: 18 patients (43.90%) underwent surgical resection, 2 patients (4.88%) underwent surgical resection followed by chemoradiation therapy, and 21 patients (51.22%) underwent chemoradiaton therapy.

Approval for the study was granted by the local Ethics Committee, and all procedures were performed in accordance with the Helsinki Declaration of 1975, as revised in 2000.

Statistical analysis

Data were analyzed using the SPSS 20.0 software package (SPSS Inc., Chicago, IL, USA). Results are reported as means ± standard deviation. Data conformation to the normal distribution was evaluated with the Kolmogorov-Smirnov test. Since normal distribution was confirmed in both HGF and CK18 levels, differences between the groups were analyzed with the independent samples t-test and the one-way ANOVA. P < 0.05 in a 95% confidence interval was accepted as being statistically significant.

RESULTS

The demographic characteristics and serum CK18 and HGF levels of both groups are displayed in Table 1. No statistically significant difference was observed between the patient and control groups with respect to age or gender. Results showed significantly elevated serum CK18 levels in patients with esophageal cancer (2.53 ± 1.33 ng/mL) as compared to healthy controls (0.34 ± 0.23 ng/mL) (P < 0.001). Similarly, serum HGF levels were significantly higher in the patient group (1.37 ± 0.63 ng/mL) than in the control group (0.41 ± 0.29 ng/mL) (P < 0.001).

Table 1. Demographics and serum CK18 and HGF levels in the patient and control groups.

	Patient group (N = 41)	Control group (N = 39)	P value
Age (years, means ± SD)	63.54 ± 10.88	61.82 ± 9.87	0.234
Gender (N, female/male)	21/20	20/19	0.83
CK18 (ng/mL)	2.53 ± 1.33	0.34 ± 0.23	<0.001
HGF (ng/mL)	1.37 ± 0.63	0.41 ± 0.29	<0.001

The statistical evaluation of serum CK18 and HGF levels and clinicopathological features are shown in Table 2. When serum CK18 and HGF levels were evaluated according to the clinicopathological features, we found that different clinicopathological features correlated with different serum CK18 and HGF levels, with the exception of node stage (Table 2). It was observed that serum HGF and CK18 levels were significantly higher in patients with cancer metastasis as compared to those with no metastasis. In addition, higher serum HGF and CK18 levels were found in patients at the T4 tumor stage as compared to those at the T3 tumor stage. We did not include data from two patients at the T2 tumor stage in our statistical analysis, as the variances were non-homogeneous.

Table 2. Serum CK18 and HGF levels for each disease parameter in ESCC patients.

Disease parameters	CK18 (ng/mL)	P value	HGF (ng/mL)	P value
Tumor stage		0.001		<0.001
T_3 (N = 24)	1.89 ± 1.11		1.09 ± 0.39	
T_4 (N = 15)	3.42 ± 1.20		1.80 ± 0.58	
Metastatis stage		<0.001		<0.001
M_0 (N = 13)	1.06 ± 0.23		0.78 ± 0.15	
$M_{1a/1b}$ (N = 28)	3.21 ± 1.06		1.65 ± 0.47	
Node stage		0.566		0.338
N_0 (N = 3)	2.17 ± 1.53		1.16 ± 0.44	
N_1 (N = 38)	2.49 ± 1.32		1.41 ± 0.67	
Stage		<0.001		<0.001
Stage III (N = 25)	0.99 ± 0.23		0.73 ± 0.14	
Stage IV (N = 16)	3.09 ± 1.01		1.60 ± 0.42	

Pearson correlation analysis showed a strong positive correlation between serum CK18 and HGF levels in the patient group (P < 0.001, r = 0.522) (Figure 1). The correlation coefficients and P values for all the parameters are given in Table 3.
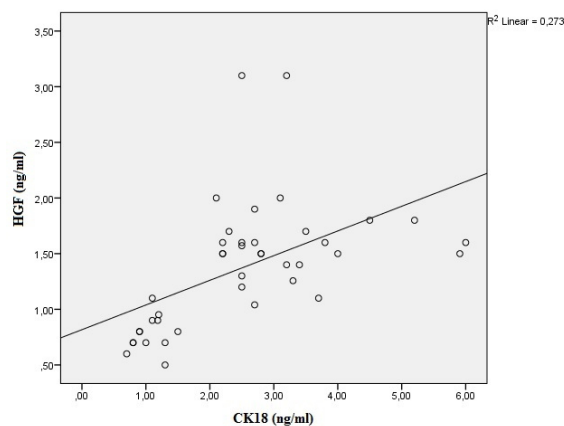

Figure 1. Correlation between serum HGF and CK18 levels in ESCC patients.

Table 3. Correlations between serum CK18/ HGF and disease parameters.

Disease parameters	CK18	HGF
Metastatis stage	(P < 0.001, r = 0.753)	(P < 0.001, r = 0.721)
Node stage	(P = 0.020, r = 0.363)	(P = 0.009, r = 0.400)
Tumor stage	(P = 0.014, r = 0.379)	(P = 0.002, r = 0.463)
Stage	(P < 0.001, r = 0.677)	(P < 0.001, r = 0.639)

P = test statistic P value; r = correlation coefficient.

DISCUSSION

ESCC is a type of cancer that exhibits high mortality rate; by the time of disease diagnosis, the cancer would have already reached advanced stages. Therefore, the identification and usage of clinical biomarkers for ESCC is an important field of research.

In this study, we aimed to analyze serum CK18 and HGF levels in ESCC patients, and to evaluate the relationship between these biomarkers and disease activity. Our results showed that serum CK18 and HGF levels were significantly higher in ESCC patients with advanced tumor stages (pT3 and pT4) as compared to healthy controls. Furthermore, serum CK18 and HGF levels also correlated with each other. CK18 and HGF levels were significantly higher in advanced stages of ESCC, which include manifestation of distinctive clinicopathological features such as metastasis, advancing tumor stages, and advancing ESCC stages. In addition, as serum CK18 and HGF levels were found to be associated with cancer metastasis, evaluation of serum HGF and CK levels in patients during biopsy may be beneficial for predicting metastasis risk or tumor staging of ESCC patients without using radiological imaging techniques.

CK18 is highly expressed in ESCC patients, and studies have shown that upregulated expression of this protein is associated with poor diagnosis in squamous cell carcinomas (Fillies et al., 2006). CK18 is normally expressed in non-cancerous glandular, transitional and hepatocyte epithelium, but not in squamous stratified epithelium (Trask et al., 1990; Schüssler et al., 1992). Therefore, the CK18 protein may serve as a potential biomarker for the diagnosis and prognosis of ESCC. In a large immunohistochemical study conducted on 210 resected specimens from ESCC patients, CK18 and its co-expressed complementary subunit CK8 were reported to be highly expressed in cancerous squamous epithelium of the esophageal glands; this expression was correlated with progression of ESCC. Researchers have suggested that CK18 may be useful in selecting treatment strategies for ESCC (Makino et al., 2009). Another large study conducted on 219 gastric or gastro-esophageal cancer patients (97 patients who received pre-operative platinum-based chemotherapy and 122 patients who received no neoadjuvant chemotherapy) showed that CK18 was commonly (92.6%) expressed in gastro-esophageal tumors (Fareed et al., 2012). Micrometastasis is considered a key step in cancerogenesis, and the detection of micrometastasis is crucial in deciding whether or not adjuvant therapy is necessary following surgery (Noguchi et al., 2003). Thus, the CK18 protein is an important factor in determining the progression of ESCC.

Studies have indicated that factors produced by the extracellular matrix, which alter the tumor microenvironment, may change carcinoma behavior and affect its prognosis. Cancer-associated fibroblasts have such a role in tumorigenesis (Bhowmick et al., 2004), and are present in various types of squamous cell carcinomas including ESCC (Iwazawa et al., 1996; Okawa et al., 2007). Previous studies have demonstrated that cancer-associated fibroblasts isolated from mammary and prostate tumors promote tumorigenesis in normal epithelium.

Conversely, fibroblasts from normal tissues converted tumorigenic skin and prostate epithelial cells into morphologically benign lesions (Cooper and Pinkus, 1977; Olumi et al., 1999).

HGF is a mesenchymal cytokine, and acts as a potent mitogen and pro-motility agent for epithelial cells by activating mitogenesis, morphogenesis, and angiogenesis (Bussolino et al., 1992; Grant et al., 1993). The term scatter factor, another term for HGF, was named for the ability of HGF to induce hepatocyte scattering, similar to a mitogen (Nakamura et al., 1986; Stoker et al., 1987). This angiogenic cytokine exerts its effects on epithelial tissues through a paracrine mode of action. c-Met is the HGF receptor on epithelial cells, and belongs to the tyrosine kinase family of receptors. Aberrant HGF/cMET activation is involved in various types of carcinomas including ESCC. Serum HGF levels have been evaluated and reported to be related to poor prognosis in prostate cancers (Ren et al., 2005), lung cancers (Takada et al., 1995) and ESCC (Grugan et al., 2010). HGF levels have also been associated with advanced tumor stage and metastasis in ESCC patients, and *in vitro* studies have demonstrated that HGF plays a role in promoting invasion, migration, and metastasis of ESCC cells (Grugan et al., 2010). However, these effects could not be determined in normal epithelial cells, possibly due to the absence of c-Met on normal epithelial surface. It has been suggested that inactivating HGF using antibodies and/or inhibiting the HGF/c-Met signaling pathway may be a potential therapeutic approach for regulating the angiogenesis and metastasis of tumorigenic tissues (Grugan et al., 2010). There are promising targeted therapy strategies that have demonstrated favorable results in both completed and ongoing preclinical studies (Hack et al., 2014). The results of these studies reveal the need for biomarkers that are not only used for diagnosis and prognosis, but also for determining patients who are more prone to benefit from targeted therapies.

Results from the study show that CK18 and HGF may be valuable biomarkers that can be used to determine pathological staging, disease prognosis, as well as to monitor treatment responses and disease recurrence in ESCC. To the best of our knowledge, this is the first study evaluating serum levels of CK18 and HGF in patients with ESCC. However, our preliminary study lacks patients with early stage tumors. Further studies need to be conducted on ESCC patients with both early and advanced tumor stages in order to fully elucidate the mechanisms of action of CK18 and HGF on the development and progression of ESCC. Determining cut-off levels for different disease stages may lead to clinical use of serum CK18 and HGF levels as disease monitoring parameters.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

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