

Review Article

Cytokines Association with Clinical and Pathological Changes in Esophageal Squamous Cell Carcinoma

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Carcinogenic transformation of cells in esophageal squamous cell carcinoma (ESCC) is characterized on molecular level by, among other things, changes in protein expression. Among all proteins related to inflammation, cytokines may be implicated as possible biological markers of esophageal cancer. These biomarkers, near imaging techniques, may be helpful in diagnosis and monitoring therapy in ESCC patients. This review demonstrates findings of researches on dysregulation of cytokines in ESCC and their clinical and pathological implications. Articles on cytokines were selected according to the following criteria: (i) the study was performed at protein level, (ii) the differences in cytokines expression or concentration were detected in tissues or serum from ESCC patients, (iii) the alterations of cytokines levels were detected by: immunohistochemistry (IHC), western blot (WB) and enzyme-linked immunosorbent assay (ELISA). Members of VEGF family seem to play an essential role as potential markers in ESCC. The results of all cytokines researches are promising but further studies are necessary to establish the biological significance of these peptides in ESCC, their potential usefulness for early diagnosis, pre- and postoperative prognosis and monitoring of the respond to chemo- and radiotherapy of cancer patients.

1. Introduction

Cancers of esophagus are placed among the most common malignancies worldwide, being the eighth leading cause of cancer-related deaths [1]. Esophageal squamous cell carcinoma (ESCC) is a dominant histological type of esophageal malignancies [1, 2]. This cancer is usually detected in the advanced stage of disease, when regional and/or distant metastases are presented [2, 3]. For this reason, poor prognosis and survival prediction for ESCC patients are observed. In ESCC, dissemination of disease at diagnosis is favored by lack of serosa and rich structure of submucosal lymphatic vessels [1–3]. Because of that most ESCC patients have invisible micrometastases in tumor region at the time of diagnosis.

Surgical resection of tumor is an essential method of ESCC treatment. Then, radio- and chemotherapy are complementary methods of treatment [4, 5]. Development of clinical observations related to such imaging techniques as endoscopy, computer tomography (CT), magnetic resonance, and positron emission tomography, helps in detection of dysplasia or neoplasia in the esophagus. These techniques

are usually very accurate in the determination of methods of cancer treatment. However, it is occasionally demonstrated in the time of operation, that progression of cancer is more extensive and treatment is generally only palliative.

Two most important risk factors for ESCC are cigarette smoking and alcohol consumption [6]. They contain various chemical carcinogens, which stimulate inflammatory reactions, induce oxidative stress parameters, destabilize genome, change enzymes function, and stimulate angiogenesis [6]. In recent years, it has been demonstrated that carcinogenic transformation of cells is characterized on molecular level by, among other things, changes in protein expression [7–9]. These proteins can be markers of early progression of esophageal cancer. New biomarkers, near imaging techniques, may be helpful in diagnosis and monitoring therapy in ESCC patients. Identification of biological markers with the use of easy and noninvasive techniques to measure them becomes crucial. A lot of researches, conducted by methods of enzyme-linked immunosorbent assay (ELISA), western blot (WB), immunohistochemistry (IHC), and proteomic or flight-mass spectrometry approaches, detect dysregulation of

protein level in serum and/or tumor tissues in ESCC patients. Study on relationship between protein alterations and clinical and pathological parameters can disclose the potential role of these molecules as ESCC biomarkers [7–10].

Among all proteins related to inflammation, cytokines play an important role in cancer development and progression and may be implicated as possible biological markers of esophageal cancer.

2. Search Strategy and Criteria of Selection

Published results for this review were found by searching of PubMed, US National Library of Medicine, and National Institute of Health (<http://www.ncbi.nlm.nih.gov/pubmed>). In the search, I used the following phrases: “cytokines in esophageal squamous cell carcinoma” (110 articles), “interleukins in esophageal squamous cell carcinoma” (24 articles), “growth factors in esophageal squamous cell carcinoma” (292 articles), “esophageal squamous cell carcinoma, review” (147 articles), “cytokines in esophageal cancer, review” (67 articles), “angiogenesis in esophageal squamous cell carcinoma” (80 articles), and “VEGF in ESCC” (85 articles).

I selected articles on cytokines according to the following criteria: (i) the study was performed at protein level, (ii) the differences in cytokines expression or concentration were detected in tissues or serum from ESCC patients, and (iii) the alterations of cytokines levels were detected by immunohistochemistry (IHC), western blot (WB), and enzyme-linked immunosorbent assay (ELISA).

After selection, data from 49 articles and information about 22 cytokines related to clinicopathological parameters of ESCC patients were taken into consideration in this review.

3. Biological Role of Cytokines and Growth Factors

Cytokines belong to the group of soluble proteins with low molecular weight. They play a role of intercellular mediators, setting cell growth processes and participating in their differentiation, migration, and apoptosis [11, 12]. They are secreted by many types of specific cells of the innate and adaptive immune systems. Cytokines, through their specific receptors, have an influence on a variety of cellular activities.

They play an important role in immunity, inflammation, repair, tissue homeostasis, and hematopoiesis [11]. Cytokines are characterized by pleiotropism, redundancy, synergism, and antagonism effects [13, 14]. The group of cytokines includes currently many factors with diverse origin and various functions. Therefore, clear classification of these peptides is difficult to implement. Structurally, they include interleukins (IL), interferons (IFNs), chemokines (as IL-8), growth factors (such as tumor growth factor-beta (TGF- β), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), and fibroblast growth factor (FGF)), tumor necrosis factor (TNF) family, hematopoietic cytokines,

and others. Functionally, cytokines are divided into proinflammatory factors (such as IL-1, IL-6, IL-8, IL-18, IFN- γ , TNF- α , TNF- β , and FasL) and anti-inflammatory factors (such as IL-4, IL-10, and TGF- β), [11–14].

Several inflammatory cytokines have been implicated to participate in different molecular mechanisms which lead to carcinogenesis [14, 15]. It is common knowledge that malignant transformation process is strongly connected with the altered responses of cytokines expression [13, 15]. Cytokines play also an important role in tumorigenesis and angiogenesis stimulation and metastasis induction [13–15].

4. Cytokines in SCC of Esophagus

Tumor microenvironment contains not only cancerous cells, but also fibroblasts, endothelial cells, immune cells, and cytokines which play a key role in the control of communication between these types of cells [8, 12, 14–16]. Under certain conditions, in the first phase of cancer development, endogenous cytokines may stimulate host immune response against tumor cells. However, recent data indicate that the majority of these factors may induce immune dysfunctions and contribute to tumor growth, progression, metastasis, and host immunosuppression [8, 12, 13]. For this reason, alterations in microenvironment of ESCC may have a clinical significance in cancer development. As in other malignant tumors, also esophageal cancer cells suppress host immune defense by secreting a number of immunosuppressive factors [8–10, 12].

The cytokine network of ESCC is rich in proinflammatory cytokines, growth factors, and chemokines. I demonstrated in this study that twenty-two cytokines are associated with clinical and pathological parameters and survival rates in ESCC (Table 1). Prominent ESCC relevant cytokines are VEGF-A, VEGF-C, VEGF-D, bFGF, HGF, MIF, TGF- β , IL-6, IL-8, and FasL as well as midkine, IL-18, PDGF-BB, CTGF, and CXCL12 [17–66]. High expressions of VEGF family members, HGF, bFGF, have been detected in ESCC tumor tissue, suggesting their potential role in tumor growth, angiogenesis and metastasis processes [17–53]. Also serum VEGF-A, C, D members, IL-8, IL-6, IL-18, TGF- β , HGF, FasL, PDGF-BB, and midkine concentrations were associated with tumor progression, lymph node metastasis, and distant metastasis parameters [54–66].

Probably functions of ESCC-derived cytokines are presented in Table 2. MIF is related to regulation growth processes, and IL-1 β , IL-6, and IL-8 are associated with regulation transcription. IL-1 β and IL-6 are stimulators of proinflammatory cytokine production. FasL induces apoptosis of activated lymphocytes in host immune defense system and in consequence stimulates progression of cancer [44, 65]. Transforming growth factor-beta (TGF- β) plays a dual role in the tumor development. In the early phase of carcinogenesis, this cytokine acts as a tumor suppressor, but later it is an inductor of tumor invasion by stimulation of extracellular matrix production, tumor cells proliferation, angiogenesis, and inhibition of host immune functions [42].

TABLE 1: Prognostic values of cytokines and growth factors in ESCC.

Cytokine	Studies [reference]	Number of patients (pTNM)	Methods of detection	Protein level (% of positive)	Relationship between pTNM parameters and MVD	Survival analysis Univariate/multivariate	Correlation with other factors
VEGF-A	Koide et al., 1999 [17]	52 (I-III)	IHC	+ (58%)	MVD+, N+, M+	$P < 0.05/-$	-
	Shih et al., 2000 [18]	117 (I-III)	IHC	+ (31%)	T+, N+, MVD+	$P = 0.006/P = 0.046$	-
	Mukherjee et al., 2003 [19]	55 (I-III)	IHC	+ (69%)	N+	NS	-
	Matsuyama et al., 2005 [20]	215 (I-III)	IHC	+ (53%)	-	-	HIF-1 α
	Choi et al., 2006 [21]	51 (I-III)	IHC	+ (41%)	NS	$P = 0.06/P = 0.007$	-
	Luo et al., 2011 [22]	134 (III)	IHC	+ (44%)	-	$P = 0.003/-$	-
	Kleespies et al., 2004 [23]	Review	IHC, ELISA	+	TNM+, T+, N+, M+, MVD+	$P = 0.007/P = 0.198$	-
	Kleespies et al., 2005 [54]			+		$P < 0.001/P = 0.001$	-
	Ren et al., 2005 [24]	72 (II-IV)	IHC, ELISA	+	-	-	MIF
	Shimada et al., 2001 [55]	96 (I-IV)	ELISA	+	TNM+, T+, N+, M+	$P < 0.001/P < 0.001$	MIF
Ren et al., 2005 [41]	149 (I-IV)	ELISA	+	-	-	HGF	
Krzystek-Korpacka et al., 2007 [56]	25 (II-IV)	ELISA	+	T+	-	-	
VEGF-C	Kitadai et al., 2001 [25]	60 (I-III)	IHC	+	TNM+, T+, N+, M+, MVD+	-	-
	Noguchi et al., 2002 [26]	71 (I-III)	IHC	+	NS	-	-
	Kimura et al., 2003 [27]	112 (I-III)	IHC	+ (39%)	TNM+, T+, N+	-	-
	Byeon et al., 2004 [28]	34 (I-III)	IHC	+ (77%)	TNM+, T+, N+	-	COX-2
	Katsuta et al., 2005 [29]	48 (I-III)	IHC	+ (60%)	NS	NS	HIF-1 α
	Matsumoto et al., 2006 [30]	87 (I-III)	IHC	+	T+, L+, MVD+	-	-
	Han et al., 2007 [31]	40 (I-III)	IHC	+ (60%)	NS	-	-
	Tao et al., 2012 [32]	90 (I-III)	IHC	+ (83%)	TNM+, T+, N+	-	MMP-1
	Song et al., 2012 [33]	99 (I-III)	IHC	+ (50%)	-	$-/P < 0.001$	-
	Zhang et al., 2012 [34]	49 (I-III)	IHC	+ (73%)	N+	-	-
	Zhang et al., 2012 [35]	48 (I-III)	IHC	+	TNM+, T+, N+, M+	$P < 0.05/-$	-
	Krzystek-Korpacka et al., 2007 [57]	70 (II-IV)	ELISA	+	TNM+, N+, M+	$P < 0.05/-$	Midkine
	Krzystek-Korpacka et al., 2007 [56]	25 (II-IV)	ELISA	+	T+	-	-
	Kimura et al., 2008 [58]	80 (I-III)	ELISA	+	N+	$P = 0.0183/P = 0.0069$	-
	Kozlowski et al., 2010 [59]	110 (I-IV)	ELISA	+	TNM+, T+, N+, M+	$P = 0.001/NS$	-

TABLE 1: Continued.

Cytokine	Studies [reference]	Number of patients (pTNM)	Methods of detection	Protein level (% of positive)	Relationship between pTNM parameters and MVD	Survival analysis Univariate/multivariate	Correlation with other factors
VEGF-D	Tzao et al., 2008 [36]	85 (I-III)	IHC	+ (66%)	T+, N+	$P = 0.001/P = 0.035$	—
	Kozlowski et al., 2010 [59]	110 (I-IV)	ELISA	+	TNM+, T+, N+	$P = 0.08/NS$	—
TGF- β	Fukai et al., 2003 [37]	80 (I-III)	IHC, ELISA	+ (36%)	T+	NS/NS	—
	Diakowska et al., 2011 [60]	115 (I-IV)	ELISA	+	T+	—	VEGF-A
IL-1 β	Chen et al., 2012 [38]	147 (I-III)	IHC, WB	+	TNM+	$P < 0.0000/P = 0.0003$	NF- κ B
IL-2	Mahmoodi et al., 2008 [66]	40 (I-III)	ELISA	—	—	—	—
IL-6	Oka et al., 1996 [39]	50 (I-IV)	IHC, ELISA	+ (54%)	T+	—	CRP, albumin, CRP
	Fujiwara et al., 2011 [40]	16 (III-IV)	IHC	+	T+	—	—
IL-8	Ren et al., 2005 [41]	149 (I-IV)	ELISA	+	—	—	HGF, VEGF
	Ren et al., 2005 [24]	72 (II-IV)	ELISA	+	—	—	—
	Krzystek-Korpacka et al., 2008 [61]	70 (I-IV)	ELISA	+	N+, M+	—	VEGF-C
IL-12	Diakowska et al., 2006 [62]	41 (I-IV)	ELISA	+	TNM+	—	IL-18
IL-18	Diakowska et al., 2006 [62]	41 (I-IV)	ELISA	+	TNM+, T+, N+	—	IL-12
PDGF-BB	Krzystek-Korpacka et al., 2011 [63]	84 (II-IV)	ELISA	+ (76%)	TNM+, N+, M+	—	VEGF-A, VEGF-C
HGF	Ren et al., 2005 [41]	149 (I-IV)	ELISA, IHC, WB	+	TNM+, M+	$P = 0.0137/P = 0.0001$	VEGF, IL-8
	Takada et al., 1995 [64]	37 (I-III)	ELISA	+ (97%)	NS	NS	—
CTGF	Zhou et al., 2009 [42]	80 (I-III)	IHC, WB	+ (49%)	NS	$P = 0.018/P = 0.028$	Thrombospondin (THBS1), cysteine-rich protein 61 (Cyr61)
CXCL12	Sasaki et al., 2009 [43]	214 (I-IV)	IHC	+ (54%)	TNM+, N+	$P = 0.006/P = 0.262$	—
FasL	Shibakita et al., 1999 [44]	106 (I-III)	IHC	+ (49%)	LV+	NS/NS	—
	Takikita et al., 2010 [45]	313 (I-III)	IHC	+ (4%)	NS	$P = 0.45/NS$	—
	Kozlowski et al., 2007 [65]	100 (I-IV)	ELISA	+	TNM+, N+, M+	$P = 0.52/NS$	—
aFGF	Sugiura et al., 2007 [46]	79 (I-III)	IHC	+ (59%)	NS	$P = 0.06/NS$	bFGF, FGF-R
bFGF	Han et al., 2005 [47]	79 (I-IV)	IHC	+ (72%)	TNM+, T+, N+, MVD+	$P = 0.021/—$	HPS
IFN- γ	Mahmoodi et al., 2008 [66]	40 (I-III)	ELISA	—	—	—	—

TABLE 1: Continued.

Cytokine	Studies [reference]	Number of patients (pTNM)	Methods of detection	Protein level (% of positive)	Relationship between pTNM parameters and MVD	Survival analysis Univariate/multivariate	Correlation with other factors
IGFBP7	Kashyap et al., 2013 [48]	140 (I-III)	IHC	+ (91%)	—	—	—
IGF-II	Imsumran et al., 2007 [49]	100 (I-III)	IHC	+ (50%)	TNM+, T+, N+, M+	$P = 0.0028/P = 0.0027$	—
Midkine (HBGF)	Ren et al., 2006 [50]	66 (I-III)	IHC	+ (56%)	NS	NS	—
	Shimada et al., 2003 [51]	93 (I-IV)	ELISA, IHC	+ (61%)	NS	$P < 0.001/P = 0.004$	—
	Krzystek-Korpacka et al., 2007 [57]	14 (I-III)	ELISA, IHC	—	NS	—	—
MIF	Krzystek-Korpacka et al., 2007 [57]	70 (II-IV)	ELISA	+	TNM+, T+, N+, M+	—	VEGF-C
	Ren et al., 2005 [24]	72 (II-IV)	IHC	+	—	—	VEGF
	Xia et al., 2005 [52]	52 (I-III)	ELISA	+	N+, MVD+	$P = 0.03/NS$	VEGF, IL-8
	Zhang et al., 2013 [53]	136 (I-III)	WB IHC	+ (96%) +	— NS	— $P < 0.033/P < 0.011$	— CXCR4

IHC: immunohistochemistry, ELISA: enzyme-linked immunosorbent assay, WB: western blot, pTNM: pathological TNM staging system, +: overexpression, -: lower level versus control, NS: not statistically significant, TNM+: progression of cancer stage, T+: tumor progression, N+: lymph node metastasis, M+: distant metastasis, MVD+: microvessel density progression, LV+: lymph vessel invasion, -: not tested, HIF-1 α : hypoxia inducible factor-1 alpha, NF- κ B: nuclear factor-kappa B, HPS: heparinase, CRP: C-reactive protein, and COX-2: cyclooxygenase-2; other names are in Abbreviations.

Although the majority of cytokines are known to contribute to cancer growth and metastasis, others, such as IL-2, IL-12, IL-23, IL-27, and IFN- γ , may stimulate antitumor immune responses by different molecular mechanisms [67].

5. Cytokines as Markers of ESCC Presence

Histological changes in ESCC development include mild-to-severe epithelial dysplasia, carcinoma *in situ*, and invasive cancer [2]. The mechanisms for the pathogenesis of esophageal cancer are still unclear. Molecular studies have revealed that genetic alterations, along with alcohol consumption and cigarette smoking, are responsible for the sequence of pathological changes in squamous epithelium of esophagus [2, 6, 68, 69]. Early detection of this type of cancer may be the most useful means in effective treatment of ESCC patients. Because of that, searching for the alternations in cytokines levels, which may be associated with tumorigenesis, is necessary.

In this review, twenty-two cytokines demonstrated abnormal levels in ESCC patients (Table 1). Among these peptides, in twenty significantly higher levels [17–65] and only in IL-2 and IFN- γ -significantly lower levels [66] were reported.

Investigations with IHC studies indicated higher expression of chosen cytokines in esophageal carcinoma tissue in comparison to the normal tissue. It suggests that upregulation of these peptides is involved in tumor growth and progression. Studies on VEGF-A, VEGF-C, TGF- β , IL-1 β , IL-6, HGF, CTGF, CXCL12, FAS-L, a-FGF, bFGF, IGF1, IGF-II, midkine, and MIF tumor expressions have demonstrated relationship between cytokines production and cancer progression [17–53]. However, biological function of these cytokines in tumor cells and tumor microenvironment is different. On the one hand, the expression of cytokines in tumor cells promotes tumor growth and stimulates oncogenic transformation; on the other hand, production of these peptides in immune cells of tumor microenvironment may contribute also to antitumor immune response [58].

The majority of studies analyzed the expression of cytokines in tissue specimens from esophagus by using IHC method. However, these researchers examined expression of cytokines in tumor tissues both, in the early and in the advanced TNM stages of cancer. There is lack of study, which would analyze cytokines expression only in the early stage of ESCC. Absence of this type of researches is a result of high invasiveness of ESCC, and therefore there is a small group of patients with early stage of cancer.

Serum cytokines correlate positively with tumor stage, angiogenesis, and metastasis. The biological significance of circulating cytokines in esophageal cancer is currently unknown. One of the hypotheses suggests that high levels of some cytokines in serum may be related to induced apoptosis of activated lymphocytes, making facilitate tumor cells progression and metastasis easier. This inactivation of the host immune surveillance may be important for circulation of cancer cells in blood and lymph [65]. Several ELISA studies, including ours, showed significantly higher VEGF-A,

TABLE 2: Cellular functions of cytokines relevant to ESCC.

Function	Cytokine [references]
Immune suppression	TGF- β [13, 16, 37, 60]
Growth regulation	MIF [13, 24, 52, 53]
Transcription regulation	IL-1 β [13, 38], IL-6 [39, 40], IL-8 [25, 41, 61]
Inflammatory cytokines secretion	IL-1 β [11, 13, 38], IL-6 [11, 13]
Apoptosis negative regulation	FasL [44, 45, 65]
Host immune stimulation (Th1 response)	IL-2 [66], IFN- γ [66], IL-12 [62, 67], IL-18 [62, 67]
Angiogenesis stimulation	VEGF-A [7, 8, 13, 17–24, 41, 54–56], VEGF-C [7, 13, 25–35, 56–59], VEGF-D [13, 36, 59], IL-8 [13, 24, 41, 61], HGF [13, 41, 64, 74], bFGF [7, 9, 13, 46, 47], PDGF-BB [13, 63], MIF [13, 24, 52, 53]
Metastasis induction	VEGF-A [8, 9, 13, 17–24, 41, 54–56], VEGF-C [9, 13, 25–35, 56–59], VEGF-D [13, 36, 59], bFGF [13, 46, 47], HGF [41, 64, 74], midkine [50, 51, 57]

VEGF-C, VEGF-D, TGF- β , IL-6, IL-8, IL-12, IL-18, PDGF-BB, HGF, FasL, MIF, and midkine concentrations in serum of ESCC patients [54–65]. Relations between VEGF-A, VEGF-C, VEGF-D, IL-12, IL-18, PDGF-BB, HGF, FasL, and midkine serum concentrations and stages of cancer progression were found [35, 37, 41, 55–65].

Analysis of IFN- γ and IL-2 revealed strongly decreased serum level of these factors in ESCC patients [66]. Both cytokines are important inducers of Th1-associated inflammatory responses and act against cancer development [13, 66].

Based on the IHC and ELISA research, I suggest that VEGF-A, VEGF-C, and HGF could be useful biomarkers for clinical diagnosis of ESCC presence, but not for early detection of esophageal cancer [17–35, 41, 54–59, 64].

It has been observed that serum levels of VEGF-A, VEGF-C, VEGF-D, and TGF- β were elevated in ESCC patients and decreased significantly after surgical treatment [59, 60]. The serum assay of these cytokines may be helpful for monitoring treatment efficacy in patients with ESCC.

6. Cytokines as Proangiogenic Factors

Angiogenesis process is essential for tumor growth and progression in ESCC [7, 13, 70–73]. A growing tumor needs

an extensive vascular network to provide oxygen and nutrients. The construction of new intratumoral blood vessels enables cancer cells to do circulation and distant metastasis. The formation of new capillary blood vessels requires different successive steps, which are mediated and regulated by a wide range of angiogenic factors, such as proangiogenic enzymes, adhesion molecules, endothelial-specific receptors, proangiogenic cytokines, chemokines, and growth factors [1, 71–73].

Angiogenic factors may act directly on vascular endothelial cells, stimulating their activity, differentiation, proliferation, and migration. The following cytokines are present in ESCC: the VEGF family members, bFGF and IL-8 [7, 71].

From the above-mentioned factors, the VEGF family members seem to play the most important role in this process. VEGF-A (known also as VEGF) contributes to tumor angiogenesis and tumor growth. This growth factor protects endothelial cells from apoptosis and plays an important role in maintenance of the vascular system balance [23, 71]. In clinical studies, high expression of VEGF-A was found to be associated with tumor progression [18, 23, 55, 56], and especially, with local metastases to lymph nodes [17–19, 23, 54, 55]. Two studies showed that VEGF-A overexpression is associated with new vascular network formation [18, 23].

Most of researchers reported that VEGF-C and VEGF-D are implicated in the development of new lymphatic vessels [25, 27, 28, 30, 32, 34–36, 57–59]. Significant relationship between VEGF-C protein expression or serum concentration and presence of lymph node metastases was demonstrated in ten articles [25, 27, 28, 30, 32, 34, 35, 57–59]. In eight articles, authors showed correlation between VEGF-C and depth of tumor invasion [25, 27, 28, 30, 32, 35, 56, 59]. Significant relation between serum or tissue VEGF-D level and lymph node metastasis has been presented in two studies [36, 59]. In these articles, authors demonstrated also the relationship between VEGF-D and stages of tumor progression. Present results suggest the possibility that cytokines forming VEGF family play a crucial role in lymphangiogenesis as independent negative prognostic factors.

Another angiogenic factor bFGF is also reported to have prognostic impact on ESCC angiogenesis. Han et al. [47] have shown by IHC study that bFGF expression is significantly associated with microvessel formation and also with primary tumor progression and lymph node metastasis.

There is also second group of cytokines, which induce proangiogenic cytokines production. These include IL-1 β , IL-6, IL-8, MIF, HGF, PDGF-BB, and TGF- β .

IL-1 β , IL-6, and IL-8 are important in induction of signal transduction pathway, which is involved in transcription of proangiogenic cytokines [38]. They contribute to cancer development, tumor proliferation, and metastasis [38–41, 61].

MIF can induce the secretion of angiogenic factors as VEGF-A and IL-8 in tumor, but not in normal epithelial cells. This cytokine overexpression correlates with lymph node status, tumor differentiation, microvessel density, and survival of ESCC patients and also correlates positively with VEGF-A expression [24].

Investigation on tumor tissue and serum demonstrated that HGF levels are associated with stage of cancer progression and metastasis process [41, 74]. The study by Ren et al. [41] showed also that HGF stimulates the expression of VEGF-A, IL-8, and PDGF.

Another factor which plays a significant role in tumor development is TGF- β [37, 60]. The study Deng et al. [75] demonstrated that TGF- β signaling pathway is disturbed in ESCC and can contribute to tumor invasion, metastases, and patients survival. Also connective tissue growth factor (CTGF) is one of the proteins which is involved in the TGF- β signal pathway and plays an important role in activation of angiogenesis and tumor growth [75].

In nine other studies, the increase of levels of cytokines, such as IL-12, IL-18, PDGF-BB, midkine, FasL, and IGF-II, was related to a greater depth to tumor invasion, lymph node metastasis, and higher TNM stage in ESCC patients (Table 1).

Sasaki et al. [43] reported that chemokine CXCL12 is overexpressed in ESCC and this expression is associated with the lymph node metastasis.

7. Cytokines as Markers of Distant Metastasis

Angiogenesis has been linked to metastasis formation, which is the principal cause of cancer mortality [71–73, 76]. Also the biological factors which are involved in metastasis often participate earlier in cell adhesion and angiogenesis processes [9, 72].

Neoplastic cells, which might acquire the ability to invasion and metastasis, leave primary tumor and colonize new tissue in the host body. Tumor cells use a variety of mobility mechanisms for their own changes and migration. Biochemical mediators, such as cytokines, are involved in these complex processes. Seven cytokines were related to metastasis of ESCC (Table 1). Three studies demonstrated that VEGF-A and VEGF-C overexpression may play an important role in distant migration of cancer cells [17, 25, 35] while circulating levels of VEGF-A and VEGF-C were found to be significantly elevated in patients with distant metastasis in five studies [23, 35, 54, 55, 59]. These results confirmed that VEGF-A and especially VEGF-C, through induction of hyperplasia of lymph vessels, are strong factors of ESCC metastasis processes. Kleespies et al. suggest that high levels of these cytokines in serum may contribute to the growth of micrometastases at distant body regions [54].

Immunohistochemistry study by Imsumran et al. revealed that high expression of IGF-II in ESCC tumor tissue correlates with lymph node and distant metastasis [49].

Findings described by Krzystek-Korpacka et al. [61, 63] demonstrated that serum IL-8 and PDGF-BB concentrations were related to local and distant metastases.

Also levels of serum HGF correlated positively with VEGF-A and IL-8 and were associated with distant metastases [41]. Serum FasL concentration was significantly higher in ESCC patients with distant metastases, which confirmed the suggestion that FasL may induce apoptosis of activated lymphocytes, weakening host immune system and facilitating tumor metastasis.

8. Cytokines in Survival Prediction

In this paper, the criteria of evaluation of cytokines as prognostic and survival markers included univariate analysis with COX proportional hazards regression model, multivariate analysis, and Kaplan-Meier method.

In univariate analysis, 13 cytokines were presented as significant prognostic factors of ESCC patients. In the multivariate analysis, 10 cytokines became ones of the prognostic factors for long-term survival, whereas levels of three cytokines did not influence patients survival (Table 1).

However, the majority of the VEGF family studies confirm that VEGF-A, VEGF-C, and VEGF-D contribute to the aggressiveness in esophageal cancer and may be useful as the strongest marker of poor outcomes in ESCC patients. Luo et al. [22] demonstrated that combined analysis of VEGF-A expression in the primary tumor and corresponding metastatic lymph nodes could help in prediction of survival of esophageal cancer patients.

As shown in Table 1, also IL-1 β , HGF, CTGF, IGF-II, and midkine are recommended as significant predictors of survival prognosis in ESCC. MIF, the cytokine expressed in cytoplasm of tumor cells and in host immune cells in tumor microenvironments, is an adverse independent factor for disease-free survival and overall survival in ESCC patients [24, 53].

Although various cytokines have been found to be statistically significantly associated with survival of patients, using cytokines as prognostic and survival markers for clinical decision making still remains to be evaluated with consideration of the effects of false positive and false negative issues [77, 78].

9. Conclusions

Presented articles showed that some cytokines may contribute to the aggressive characteristic of ESCC and correlate with primary tumor progression, lymph and distant metastasis, and patients outcome.

Members of VEGF family seem to play an essential role as potential early markers in ESCC. Also HGF and bFGF could serve as independent prognostic markers for ESCC. The alterations in levels of proangiogenic cytokines and growth factors such as VEGF-A, VEGF-C, TGF- β , and HGF and microvessel assessment may be used for prediction of lymphangiogenesis and distant metastasis in patients with ESCC.

Although cytokines play an important role in tumor proliferation, angiogenesis, and metastasis, their mechanism of action in ESCC is not entirely explained. Further studies are necessary to establish the biological significance of cytokines in ESCC, their potential usefulness for early diagnosis, pre- and postoperative prognosis, and monitoring of the response to chemo- and radiotherapy of cancer patients.

Abbreviations

ESCC: Esophageal squamous cell carcinoma
VEGF-A: Vascular endothelial growth factor-A

VEGF-C: Vascular endothelial growth factor-C
VEGF-D: Vascular endothelial growth factor-D
aFGF: a fibroblast growth factor
bFGF: b fibroblast growth factor
TNF: Tumor necrosis factor
HGF: Hepatocyte growth factor
MIF: Migration inhibitory factor
TGF- β : Tumor growth factor-beta
IL-1 β : Interleukin-1 β
IL-2: Interleukin-2
IL-6: Interleukin-6
IL-8: Interleukin-8
IL-12: Interleukin-12
IL-18: Interleukin-18
FasL: Fas ligand
IFN- γ : Interferon- γ
IGFBP7: Insulin-like growth factor-binding protein 7
IGF-II: Insulin growth factor II
HBGF: Heparin-binding growth factor (midkine)
PDGF-BB: Platelet-derived growth factor
CTGF: Connective tissue growth factor
CXCL12: Stromal cell-derived factor 1.

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