Angiogenesis-Related Factors are Molecular Targets for Diagnosis and Treatment of Patients with Esophageal Carcinoma

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Angiogenesis plays an essential role in the growth and metastasis of esophageal carcinoma. Vascular endothelial growth factor, thymidine phosphorylase, fibroblast growth factor, midkine, and hepatocyte growth factor have been reported to be vital molecules for tumor angiogenesis. Polymorphisms in gene encoding angiogenic factors or their receptors may alter protein expression and/or activity. Increased angiogenic-factor expression and increased serum levels of these molecules were found to be associated with poor treatment response and poor prognosis. We reviewed the clinicopathological significance of angiogenesis-related molecules in patients with esophageal carcinoma. Antiangiogenic molecular-treatment strategies are also discussed. (Ann Thorac Cardiovasc Surg 2010; 16: 389–393)

Introduction

The aggressive behavior of esophageal carcinoma is often associated with angiogenesis, detected by microvessel density, vascular endothelial growth factor (VEGF) expression, thymidine phosphorylase (dThdPase/PDECGF) expression, fibroblast growth factor (FGF) expression, midkine (MK) expression, and overexpression of angiogenic factors or their receptors.[1–4] Increased serum levels of angiogenic factors, caused by increased protein expression, are found to be associated with poor treatment response and poor prognosis. Such angiogenesis-related molecules may represent a novel target in treating esophageal cancer. This review contains a compilation of available significant studies of angiogenesis in esophageal carcinoma and discusses newly developed therapies targeted at angiogenesis-related molecules.

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Overexpression of Angiogenic Factors in Esophageal Carcinoma

VEGF overexpression has been evidenced in 31%–60% of esophageal carcinoma cases.[1–3] VEGF protein expression was, in particular, significantly associated with the amount of microvessel density, distant metastasis, and survival.[5] Overexpression of VEGF protein is therefore responsible for the malignant potential in esophageal carcinoma and is a useful marker in estimating patient prognosis. High dThdPase expression and increased dThdPase activity have also been found to be associated with angiogenesis, tumor progression, and poor prognosis in esophageal carcinoma.[6,7]

Coexpression of dThdPase and p53 was assessed by immunohistochemical staining.[8] More than half the patients (52%) were positive for dThdPase expression, and a significant association between dThdPase expression and nodal involvement was observed ($P < 0.001$). Coexpression of dThdPase and p53 was strongly associated with p53 gene mutation ($P < 0.01$). Among the various prognostic variables, the overall survival was significantly worse in patients with a coexpression of p53 and dThdPase than in the other patients.

Overexpression of FGF-2 mRNA is associated with tumor recurrence and reduced survival after surgical

Table 1. Clinical impact of serum angiogenic-related biomarkers in patients with esophageal carcinoma

Serum Biomarkers	Positive Rate (%)	Prognostic Impact	Treatment Resistance	Association with TNM		
				T	N	M
VEGF[17, 18]	37	YES	YES	YES	YES	BL
dThdPase[22]	19	YES	YES	YES	BL	NO
Midkine[24, 25]	61	YES	NA	NO	NO	NO
HGF[33]	15	YES	NA	YES	YES	YES
p53-Ab[26]	31	Borderline	YES	NO	BL	NO
Platelet count[27]	21	YES	NA	YES	YES	YES

TNM, tumor depth/nodal involvement/distant metastases; VEGF, vascular endothelial growth factor; dThdPase, thymidine phosphorylase; HGF, hepatocyte growth factor.

resection of esophageal carcinoma.[9] Stromal fibroblasts also contribute to regulating extracellular matrix degradation, epithelial cell behavior, inflammation, and cancer progression. FGF receptor 2-positive fibroblasts provide a suitable microenvironment for the development of distant metastases, stimulation of cancer cell proliferation, induction of angiogenesis, enhancement of cell mobility, and promotion of the epithelial-mesenchymal transition.[10]

The ability to predict patient responses to chemoradiotherapy by analyzing pretreatment biopsy specimens would be valuable in managing esophageal carcinoma. To this end, we analyzed the expression of angiogenesis-related molecules (p53, dThdPase, and VEGF) by immunohistochemistry in patients with esophageal carcinoma prior to treatment.[11] A clinical response was observed in 69% of the patients and was negatively associated with dThdPase ($P = 0.02$) and VEGF expressions ($P < 0.01$). Multivariate analysis identified VEGF as a significant independent prognostic factor ($P = 0.03$).[11] These results suggest that the expression of angiogenic factors has predictive value for the treatment response and outcome of patients with esophageal cancer.

Genetic Polymorphism of Angiogenic Factors in Patients with Esophageal Carcinoma

Certain genetic polymorphisms of angiogenic factors may affect the angiogenic pathway and thereby the susceptibility and/or severity of cancers.[12] Functional genetic polymorphisms in the VEGF gene have been reported to correlate with VEGF promoter activity, gene expression, protein production,[13] and risks of esophageal cancer,[14] which are modulated by smoking. The other functional polymorphisms were detected in PAR-1,[15] EGF,[15] and cyclooxygenase-2 (COX-2)[16] gene. They were reported as independent prognostic markers in esophageal carcinoma

that may help to identify patient subgroups at a high risk for tumor recurrence. Germline polymorphisms of genes involved in the tumor angiogenesis pathway independently predict tumor recurrence in a homogeneous patient group with esophageal carcinoma.

Clinical Significance of Serum Levels of Angiogenic Factors in Patients with Esophageal Carcinoma (Table 1)

Pretreatment serum VEGF (S-VEGF) concentration was measured in patients with esophageal carcinoma.[17] Significant differences were observed between S-VEGF categorized by tumor size ($P < 0.01$), tumor depth ($P < 0.01$), lymph node metastasis ($P < 0.01$), and TNM stage ($P < 0.01$). Patients who achieved a partial or complete response to chemoradiotherapy showed significantly less S-VEGF than those of the nonresponder group ($P = 0.02$). Multivariate analysis indicated that S-VEGF was identified as a significant and independent prognostic factor ($P < 0.01$). S-VEGF C and D were both associated with patient survival as well as tumor progression.[18–20] S-VEGF levels increased in the angiogenesis phase of wound healing following surgical procedure.[21] Platelets are a potential source of increased S-VEGF levels; inflammatory lung complications may also be related to increased S-VEGF levels.

S-dThdPase was also measured in patients with esophageal carcinoma.[22] The dThdPase expression in esophageal cancer tissues was examined by immunohistochemistry. Statistically significant differences in S-dThdPase were observed depending on tumor size ($P < 0.01$) and tumor depth ($P < 0.01$). A high S-dThdPase was associated with dThdPase expression ($P = 0.02$), poor response ($P = 0.02$), and poor survival ($P < 0.01$). A high S-dThdPase was associated with depth of tumor invasion and a poor response to treatment.

MK, a heparin-binding growth factor, is overexpressed in esophageal carcinoma.[23] It may also play a role in tumor angiogenesis and invasion. MK expression is correlated with tumor cell differentiation. The poorer that tumor cells differentiate, the weaker MK expresses. Serum MK (S-MK) concentrations are increased in patients with esophageal carcinoma.[24,25] High S-MK was associated with tumor size, immunoreactivity, and poor survival. Multivariate analysis indicated that S-MK was an independent prognostic factor and may be a useful tumor marker for esophageal carcinoma. Increased preoperative S-MK in patients with esophageal carcinoma is associated with poor survival.

Angiogenesis-related factors, such as p53 and platelet, were also useful in evaluating tumor progression and patient survival. The high-serum p53-antibody titer group had significant association with advanced tumor stages and worse outcomes than the low titer group. High-serum p53 antibody titer was an independent prognostic factor ($P < 0.01$).[26] Platelet counts were significantly increased in patients with large tumors ($P < 0.01$), deep tumors, nodal involvement, and distant metastasis in univariate analysis. C-reactive protein level, white blood cell count, and S-dThdPase concentration were also significantly increased in patients analyzed with thrombocytosis in univariate analysis. After adjustments for tumor size and TNM factors, multivariate analysis indicated that thrombocytosis was an independent prognostic factor ($P < 0.01$).[27]

Antiangiogenic Therapy for Esophageal Carcinoma

With regard to treatment response, the mean S-VEGF of the responder group was significantly higher than that of the nonresponder group.[17] This finding supports a model in which high S-VEGF contributes to the protection of tumor vessels from chemoradiation-mediated cytotoxicity, and thereby to treatment resistance. Gorski et al. reported that ionizing radiation can induce VEGF expression in a tumor, and neutralizing the anti-VEGF antibodies were useful in blocking the action of this radiation-mediated VEGF increase.[28] Anti-VEGF therapy using anti-VEGF antibodies, anti-VEGF receptor, and a soluble form of VEGF receptor[29] may be useful in improving the subgroup of patients with high S-VEGF.

For esophageal cancer, most targeted therapy studies have been performed with EGFR inhibitors, including cetuximab, gefitinib, erlotinib, and trastuzumab. Limited experience is available with angiogenesis inhibitors, apoptosis inhibitors, and COX-2 inhibitors. Because EGF and VEGF were shown to stimulate tumor cell growth, sunitinib and vandetanib were reported to be associated with a significant dose-dependent inhibition of proliferation and enhancement of apoptosis.[30] The addition of vandetanib was associated with reductions in both VEGF- and EGF-mediated VEGFR2 phosphorylation. Coadministration of sunitinib significantly enhanced the sensitivity of cancer cells to cisplatin and irinotecan. Also, vandetanib synergistically enhanced the sunitinib-associated inhibition of cancer cell growth. Until now, targeted therapies are proven to be safe often in combination with chemoradiation, but modestly effective for esophageal cancer.[31]

COX-2 catalyzes the initial step in prostaglandin formation. It also influences apoptosis, angiogenesis, and invasion, and it plays a key role in the production of carcinogens. COX-2 inhibitors and nonsteroidal anti-inflammatory drugs could be beneficial against the development and growth of carcinoma. Encouraging results from the first clinical trials combining chemotherapy with COX-2 inhibitors in cancer patients have recently been reported.[32] Liu et al. reported on the effects of aspirin on survival following esophageal cancer resection. The five-year survival for patients on aspirin was significantly better than the control group. Survival for T2N0M0 squamous cell carcinoma patients was significantly improved with aspirin compared with control ($P < 0.01$). There was, however, no significant difference between the survival curves for T2N0M0 adenocarcinoma patients on aspirin. There were significantly more apoptotic cells in the tumors of patients who were using meloxicam. It also decreased the levels of COX-2 mRNA, COX-2 protein, and nuclear NF-κB protein, and it increased the cytoplasmic IκB protein in the cancer. The researchers concluded that the drug induces apoptosis in esophageal carcinoma in vivo by inhibiting the pathway of NF-κB-downstream regulation of COX-2. These results support the further investigation of aspirin as adjuvant therapy in some subsets of postesophagectomy patients to improve survival.[33]

Hepatocyte Growth Factor (HGF) and c-Met Signaling in Esophageal Carcinoma

HGF and its receptor, c-Met, play important roles in esophageal carcinoma development and progression. The levels of serum HGF are significantly associated with advanced tumor metastasis stage and survival. Multivariate analyses showed that serum HGF level in cell migration was an independent prognostic factor. Increased HGF serum

levels correlated positively with VEGF serum levels.[34] The inhibition of extracellular, signal-regulated kinase activation reduced HGF-mediated VEGF expression. High Met expression was also significantly associated with reduced patient survival ($P < 0.01$) and was more likely to develop distant metastases ($P < 0.01$) and local recurrences ($P < 0.01$) than low Met expression was.[35] These findings support the importance of Met expression in esophageal carcinoma and support the concept of Met tyrosine kinase inhibition as a treatment strategy.

Factors derived from the extracellular matrix create an environment conducive to tumor growth and invasion. Specialized cancer-associated fibroblasts in the extracellular matrix influence tumorigenesis. Grugan et al. reported that fibroblast HGF secretion fosters the ability of transformed esophageal epithelial cells to invade the extracellular matrix, though other unidentified factors may cooperate with HGF. Genetic modifications of both HGF in fibroblasts and its receptor Met in epithelial cells, along with pharmacological inhibition of HGF and Met, underscore the importance of this pathway in esophageal carcinoma invasion and progression.[36]

In attempts to block the malignant behavior of HGF in cancers, we isolated NK4 as a competitive antagonist against HGF-Met signaling. NK4 inhibited angiogenesis induced by VEGF and basic FGF, as well as HGF by its HGF-antagonist action by its independent HGF-antagonist action. In experimental models of distinct types of cancers, NK4 inhibited Met activation, and this was associated with the inhibition of tumor invasion and metastasis. NK4 inhibited tumor angiogenesis, thereby suppressing angiogenesis-dependent tumor growth. Cancer treatment with NK4 suppresses malignant tumors to be "static" in both tumor growth and spread.[37] NK4 is a competitive antagonist of the HGF/c-Met signal pathway. It has additional antiangiogenic activity independent of its HGF-antagonist function. NK4 is an attractive molecule for cancer therapy because of its angiogenesis-inhibitory, cancer-specific, and apoptosis-inducing effects. NK4 and neutralizing anti-HGF antibody suppressed the HGF-induced invasion. An analysis of Met-receptor tyrosine phosphorylation, proliferation, apoptosis, and blood vessels in the tumor tissues indicated that the inhibitory effect of NK4 expression, through gene transfer, may be primarily caused by the inhibition of tumor angiogenesis.[38–40]

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