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Clinical Significance of VEGF-A, -C and -D Expression in Esophageal Malignancies

Axel Kleespies Christiane J. Bruns Karl-Walter Jauch

Chirurgische Klinik und Poliklinik, Klinikum Grosshadern, Ludwig-Maximilians-Universität München, Germany

Key Words

Vascular endothelial growth factor (VEGF) · Angiogenesis · Lymphangiogenesis · Esophageal cancer · Barrett's disease

Abstract

Vascular endothelial growth factors (VEGF)-A, -C and -D are members of the proangiogenic VEGF family of glycoproteins. VEGF-A is known to be the most important angiogenic factor under physiological and pathological conditions, while VEGF-C and VEGF-D are implicated in the development and sprouting of lymphatic vessels, so called lymphangiogenesis. Local tumor progression, lymph node metastases and hematogenous tumor spread are important prognostic factors for esophageal carcinoma (EC), one of the most lethal malignancies throughout the world. We found solid evidence in the literature that VEGF expression contributes to tumor angiogenesis, tumor progression and lymph node metastasis in esophageal squamous cell carcinoma (SCC), and many authors could show a prognostic value for VEGF-assessment. In adenocarcinoma (AC) of the esophagus angiogenic properties are acquired in early stages, particularly in precancerous lesions like Barrett's dysplasia. However, VEGF expression fails to give prognostic information in AC of the esophagus. VEGF-C and -D were detected in SCC and dysplastic lesions, but not in normal mucosa of the esophagus. VEGF-C expression might be associated with lymphatic tumor invasion, lymph node metastases and advanced disease in esophageal SCC and AC. Therapeutic interference with VEGF signaling may prove to be a promising way of anti-angiogenic co-treatment in esophageal carcinoma. However, concrete clinical data are still pending.

Schlüsselwörter

Vascular endothelial growth factor (VEGF) · Angiogenese · Lymphangiogenese · Ösophaguskarzinom · Barrett-Ösophagus

Zusammenfassung

Die Wachstumsfaktoren vascular endothelial growth factor (VEGF)-A, -C und -D sind Mitglieder der proangiogenen VEGF-Glycoprotein-Familie. VEGF-A ist der wichtigste Angiogenesefaktor unter physiologischen und pathologischen Bedingungen, während VEGF-C und VEGF-D an der Lymphangiogenese, der Entwicklung und dem Wachstum von Lymphgefäßen beteiligt sind. Lokale Tumorprogression, lymphatische Metastasierung und hämatogene Tumoraussaat sind wichtige Prognosefaktoren des Ösophaguskarzinoms (EC), einer der tödlichsten Tumorerkrankungen weltweit. In der Literatur finden sich sichere Hinweise für den Einfluss der VEGF-Expression auf die Tumorangiogenese, Tumorprogression und lymphatische Metastasierung beim Plattenepithelkarzinom des Ösophagus (SCC), etliche Autoren konnten hier einen prognostischen Wert von VEGF Messungen nachweisen. Das Adenokarzinom des Ösophagus (AC) zeigt schon in frühen Entwicklungsstadien ausgeprägte Eigenschaften der Tumorangiogenese, insbesondere bei Präkanzerosen wie der Barrett-Dysplasie. Ein prognostischer Wert der VEGF-Expression beim Adenokarzinom des Ösophagus wurde jedoch bisher nicht nachgewiesen. VEGF-C und -D konnten beim SCC und dysplastischen Läsionen aber nicht in normaler Ösophagusmukosa nachgewiesen werden. Ein Einfluss von VEGF-C Expression auf Lymphgefäßinvasion, lymphatische Metastasierung und fortgeschrittene Tumorstadien ösophagealer Plattenepithel- und Adenokarzinome ist anzunehmen. Die therapeutische Hemmung der VEGF Signaltransduktion könnte eine viel versprechende antiangiogene Zusatztherapie des Ösophaguskarzinoms darstellen, wobei konkrete klinische Daten bisher noch ausstehen.

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Fax +49 761 4 52 07 14
E-mail Information@Karger.de
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Dr. med. Axel Kleespies
Chirurgische Klinik und Poliklinik, Klinikum Grosshadern
Ludwig-Maximilians-Universität München
Marchioninistrasse 15, 81377 München, Germany
Tel. +49 89 7095-0, Fax -5424
E-mail axelkleespies@aol.com

Background

Its now firmly established that the growth of new blood vessels – *angiogenesis* – is critical to both the growth and metastasis of solid tumors. The founding member VEGF-A (formerly known as VEGF) represents the best-studied element of the pro-angiogenic vascular endothelial growth factor family and plays an outstanding role in angiogenesis, growth and hematogenous spread of solid tumors (reviewed by Hicklin and Ellis [1]). Binding of VEGF-A to its receptors (VEGFR-1 and VEGFR-2) initiates an intracellular signaling pathway which results in stromal degeneration by proteolytic enzymes [2], induces endothelial sprouting through proliferation and migration of endothelial cells [3] and enhances vascular permeability for proteins, cells and plasma [1, 4]. VEGF-A protects endothelial cells from apoptosis and contributes to the maintenance of the vascular system [5]. VEGF-C and -D are relatively young members of the VEGF family, which are structurally closely related to each other and share approximately 30% identity with VEGF-A. VEGF-C and VEGF-D but not VEGF-A bind specifically to VEGFR-3 (flt-4). Its expression becomes restricted mainly to the lymphatic endothelium of adult tissues. VEGFR-3 activation induces lymphatic vessel growth – *lymphangiogenesis* – but has very little effect on blood capillaries [6–8]. In contrast to its counterpart angiogenesis, the mechanisms of lymphangiogenesis are far less well understood and the presence or absence of functional intratumoral but not only peritumoral lymphatics remains controversial [9–11]. However, in human malignancies tumor associated lymphatics are well known key components of metastatic spread. Ingrowths of tumor cells into peritumoral lymphatics and migration via lymphatics are the most common pathways of initial tumor dissemination.

Patients with esophageal carcinoma (EC) generally have a worse prognosis than those with other types of gastrointestinal tumors [12]. The pathological pN (node) status is the most powerful predictor of outcome in squamous cell carcinoma (SCC) of the esophagus [13–15]. Overall survival is poor, with 5-year survival rates of only 10–20%, whereas rates of up to 60% have been reported for node negative groups. The influence of tumor angiogenesis and pro-angiogenic molecules such as vascular endothelial growth factors on progression and recurrence of EC has been debated over the last years. The following paragraphs will review the prognostic significance of VEGF-A, -C and -D expression, in esophageal SCC, AC (adenocarcinoma) and Barrett's dysplasia, their predictive value for treatment response to surgical, radiation- or chemotherapy and new anti-VEGF strategies.

Adenocarcinoma of the Esophagus and VEGF-A

Once gastro-esophageal reflux disease (GERD) has led to the onset of metaplastic mucosa, metaplasia of the lower esophagus appears to never totally regress [16]. Therefore, Barrett's metaplasia is one of the most common precancerous lesions in the western world. In animal experiments Baatar et al. [17] could show a potential role of VEGF-A expression in esophageal ulcer healing and Auvinen et al. [18] could proove that human Barrett's esophagus is strongly neovascularized and not simply eroded. Immunohistochemistry of surgically resected tissue specimens revealed that the 'salmon-red' color of Barrett's mucosa was due to incipient angiogenesis, originating from the pre-existing vascular network in the lamina propria [18]. Lord et al. demonstrated that VEGF-A mRNA and protein expression in esophageal adenocarcinoma was significantly increased compared with Barrett's metaplasia, dysplasia and normal mucosa [19]. In Barrett's metaplasia they found the strongest VEGF-A expression in mucin containing goblet cells and microvessel density (MVD) was generally higher in AC compared with pre-neoplastic lesions. Correspondingly, Couvelard et al. found VEGF-A protein expression in metaplastic and neoplastic epithelium, which correlated significantly with vascularization [20]. The authors report stepwise increases in microvessel counts in high-grade dysplasia, intra-mucosal carcinoma and superficial carcinoma (pT1), but microvessel counts were reduced in infiltrative carcinoma and failed to provide prognostic information. Möbius et al. could demonstrate continuously increasing VEGF-A expression values in metaplasia, high-grade dysplasia, microinvasive carcinoma, and advanced carcinoma and conclude that an angiogenic switch occurs as an early event in the metaplasia-dysplasia-carcinoma sequence of Barrett's carcinoma [21]. However, VEGF-A did not correlate with patient survival or other clinicopathological data [22]. In conclusion, angiogenic properties are acquired early, particularly in pre-cancerous lesions, representing a critical step in the development of Barrett's carcinoma [23]. These findings could provide one possible explanation for the early onset of local spread and frequent recurrence of esophageal carcinoma. However, VEGF expression patterns apparently fail to give prognostic information in invasive adenocarcinoma of the esophagus.

Squamous Cell Carcinoma of the Esophagus and VEGF-A

While only a few studies have been published regarding VEGF-A expression in Barrett's dysplasia and AC, an increasing number of papers deal with VEGF-A expression in SCC of the esophagus. The main results from those studies are summarized in table 1. All of them revealed VEGF-A expression of SCC to some degree (24–93%) [24–46]. Endoscopically obtained biopsies yielded similar results [37, 40]. VEGF-A gene expression in esophageal SCC tissue could be demonstrated using RT-PCR for VEGF-A mRNA, and circulating serum-VEGF-A was detected by ELISA [29, 31, 35, 36, 38, 39, 44]. Several authors additionally assessed vascularization and could show correlations between VEGF-A protein ex-

Table 1. VEGF-A expression in human squamous cell carcinoma of the esophagus

Reference	Patients, n	VEGF-A positive tumors, %	Correlation of VEGF-A with		Prognostic value of VEGF-A	
			pathology	MVD, p	univariate, p	multivariate, p
Ahn et al., 2002 [30]	81	51	–	–	–	
Du et al., 2003 [43]	59	81	N+, G	<0.05		
Hironaka et al., 2002 [37]	73	49			0.94	
Imdahl et al., 2002 [40]	21*			+	0.021	–
Inoue et al., 1997 [36]	75	47	dI, M+, V+, G	0.0002	0.016	0.008
Kimura et al., 2004 [46]	82	62		0.002	0.002	
Kitadai et al., 1998 [34]	119	60	dI, S, L+, V+	<0.01	0.201	
Koide et al., 1999 [28]	52	58	N+, M+, V+	0.007	0.05	
Koide et al., 2001 [24]	60	58	N+, M+, L+, V+	0.0001	0.05	
Li et al., 2000 [25]	96	65	dI, N+	–	+	–
McDonnell et al., 2001 [39]	42*		–		–	
Millikan et al., 2000 [26]	27*		N+			
Mukherjee et al., 2003 [44]	55	69	N+	0.062	–	
Nagata et al., 2002 [29]	45	93				
Ogata et al., 2003 [42]	92	24	–	<0.01	0.018	0.057
Rosa et al., 2003 [41]	47	40	–		0.088	0.15
Sato et al., 1999 [32]	134	49–74	N+			
Shih et al., 2000 [27]	117	31	–	0.08	0.04	0.046
Shimada H. et al., 2001 [38]	96		pT, N+, M+		<0.001	0.001
Shimada Y. et al., 1999 [33]	116	69			0.023	0.29
Takeuchi et al., 2004 [45]	90	36	–		0.04	
Uchida et al., 1998 [35]	109	60	pT, N+, M+	0.162	0.007	0.198
Wallner et al., 2001 [31]	32		pT, N+, M+			

*Histology not specified; + = positive (p-value not available); – = negative (p-value not available); MVD = microvessel density; pT = depth of invasion according to TNM staging system; dI = depth of invasion (other than TNM); N+ = lymph node metastases; M+ = distant metastases; G = histological differentiation (grading) of tumor; V+ = vascular infiltration; L+ = lymph vessel infiltration.

pression and microvessel density (MVD) [24, 27, 28, 30, 34–36, 40, 42–44, 46]. About 67% of authors who looked for clinicopathological parameters, reported associations of VEGF-A expression and tumor stage, spread, or progression [24–26, 28, 31, 32, 34–36, 38, 43, 44]. Most of them found significant correlations with lymph node metastases, many of them also with tumor invasiveness (e.g. pT-classification) or presence of distant metastases, and some of them with vascular invasion or grade of malignancy (table 1). Nevertheless, 6 out of 18 studies could not establish any association between VEGF-A expression and those parameters [27, 30, 39, 41, 42, 45]. However, most of the studies confirm that VEGF-A contributes to the aggressive characteristics of esophageal squamous cell carcinoma, and 72% of the authors demonstrated that VEGF-A expression correlated significantly with patient outcome [24, 25, 27, 28, 33, 35, 36, 38, 40–42, 45, 46]. In conclusion, VEGF-A expression seems to be associated with local lymph node status of esophageal SCC and seems to provide prognostic value. To date it is not finally clear which of VEGF-A's functions plays the most important role in the development of local lymph node metastases, but early tumor spread to local nodes might possibly be interpreted as a secondary effect of accelerated tumor growth and invasiveness due to tumor angiogenesis and VEGF-A expression.

Serum Levels of VEGF-A

Pro- and anti-angiogenic factors have been detected in biological fluids such as blood, urine, cerebro-spinal fluid or pleural and peritoneal effusions of tumor patients [47–54]. It remains unclear whether this phenomenon represents an active expression or just a pathological passive release of molecules by apoptotic tumor cells or hematological effector cells such as macrophages or platelets during inflammatory reactions [47, 55, 56]. Raised levels of circulating VEGF-A were reported in various types of cancer [57–59] and were associated with poor outcome [54, 57, 60–64]. Shimada et al. analyzed serum VEGF-A (S-VEGF) concentration in 99 patients with primary and recurrent SCC of the esophagus [38]. S-VEGF was significantly elevated in patients with primary SCC and correlated significantly with tumor size, positive lymph nodes, distant metastases and patient survival. Multivariate analysis found S-VEGF to be an independent prognostic marker. McDonnell et al. also detected elevated S-VEGF levels in 20 SCC and 24 AC patients [39]. However, they did not find a correlation between S-VEGF levels and tumor stage or survival. This might be explained by smaller patient numbers and the combination of AC and SCC patients in one group. The authors speculate that S-VEGF might be influenced by tumor-associated macrophages.

Macrophages are potent producers of VEGF-A induced by hypoxia and transforming growth factor β1 (TGF β1) [51, 65–67]. Furthermore, alterations in S-VEGF levels might be influenced by changes in platelet numbers, and therefore measurement of plasma-VEGF-A (P-VEGF) instead of serum-VEGF-A (S-VEGF) might be more specific [68]. Nevertheless, P-VEGF levels were also found to correlate with platelet counts of EC patients [69]. Regardless of the source (growing tumor cells, apoptotic tumor cells, macrophages or platelets), raised levels of circulating VEGF-A could be detected in EC patients and high VEGF-A levels may contribute to the growth of micrometastases at distant sites [70]. However, the prognostic value of circulating VEGF-A levels remains largely undefined.

VEGF-C and -D Expression in Esophageal Carcinoma

A special feature of EC is its early lymphatic spread into local lymph nodes. The following studies examined VEGF-C and VEGF-D expression in EC and their contribution to lymphatic invasion, lymphangiogenesis and early lymph node metastases. Ishikawa et al. studied 26 cases of esophageal SCC, 26 normal tissue samples and 11 cases of esophageal dysplasia [71]. All of the carcinomas and 82% of the dysplastic samples showed VEGF-C immunoreactivity, while VEGF-D expression was observed in 65% of SCC and only 18% of the dysplastic specimens. None of the normal esophageal mucosa samples showed a positive reaction for VEGF-C or -D. The authors conclude that VEGF-C and -D might play a positive role in early stages of esophageal carcinogenesis. Noguchi et al. detected VEGF-C expression in EC cell lines, pre-operative biopsies and surgical specimens of esophageal SCC [72]. Normal and dysplastic mucosa did not exhibit VEGF-C expression. VEGFR-3 (flt-4) was mainly expressed on lymphatic endothelium, and the authors found significant positive correlations between VEGF-C expression and tumor stage, depth of the tumor, vascular- and lymphatic invasion, and lymph node metastases [72]. In contrast, Kitadai et al. contest the prognostic value of VEGF-C expression in esophageal SCC [73]. They could not find any correlation between VEGF-C immunoreactivity and clinicopathological parameters despite histological differentiation. In Barrett's disease, Auvinen et al. identified stepwise increasing VEGF-C expression during progression from Barrett's epithelium to dysplasia and to Barrett's carcinoma [18]. VEGFR-3 (flt-4) expression on lymphatic vessels was also up-regulated during development of esophageal AC and lymphatic vessels were found to actively penetrate the tumor stroma. Moreover, VEGF-C and VEGFR-3 (flt-4) expression was identified in metastatic lymph nodes [18]. In conclusion, VEGF-C and VEGF-D appear to be expressed in esophageal SCC, AC and dysplastic epithelium, while normal esophageal epithelium seems to lack VEGF-C and VEGF-D immunoreactivity. VEGF-C expression might be associated with more aggressive disease and VEGF-C seems to be involved in lymphatic tumor invasion and lymphangiogenesis in esophageal carcinoma. This mechanism would provide another explanation for the early onset of lymphatic spread in esophageal carcinomas, a phenomenon which predicts poor outcome. However, prognostic value of VEGF-C and -D expression in esophageal carcinoma remains to be evaluated.

VEGF Expression and Treatment Response

Various reports on pre-operative chemo-radiotherapy (CRT) have indicated advantages for managing esophageal carcinoma [74–77]. 3-year survival rates of more than 50% can be expected in EC patients in whom pre-operative CRT led to a complete tumor response, which is reported in 20–30% of cases [78, 79]. On the other hand, peri-operative morbidity and mortality are increased by CRT. Therefore identification of factors that could predict a response to CRT is required. Besides p53, Ki-67, and EGF-R expression [80–82], tissue oxygenation has been demonstrated to be very important for determining sensitivity to CRT [83, 84]. Only a well oxygenated cell is fully radiosensitive [85]. Microcirculation and vessel permeability are important factors for delivery of oxygen, anticancer drugs and radiosensitizers to cancer cells. Thereby the predictive value of high microvessel density could be explained. VEGF-A is known to induce vascular growth and permeability. Consequently, VEGF-A levels in EC may be critical for CRT response. However, VEGF-A expression and microvessel sprouting are also responsible for tumor nutrition, growth, local invasion and metastatic spread. Therefore, conflicting results for the prognostic value of VEGF-A and MVD during CRT could be expected. Hironaka et al. analyzed pretreatment biopsy specimens from 73 SCC patients before definitive CRT (5-FU, cisplatin, 60 Gy) [37]. VEGF-A expression was reported in 49% of the patients but did not correlate with clinicopathological parameters. In contrast to former studies [27, 86, 87], high MVD was a significant positive and independent prognostic variable for survival. This appears plausible since the authors counted microvessels with visible lumens only and conclude that lumen-MVD rather than total MVD should be a marker of the oxygenation status of the tumor. On the other hand, high vascular density does not necessarily indicate high blood flow, tissue oxygenation or drug delivery. A non-functional structure of the immature tumor vasculature may result in an impaired blood flow [88], and the interstitial fluid pressure may rise as a consequence of increasing vascular surface and permeability [89]. These two mechanisms may result in reduced drug delivery and tissue oxygenation and finally resistance to CRT. Correspondingly, Imdahl et al. suggest that esophageal tumors (SCC and AC) with low VEGF-A expression respond better to CRT [40, 89]. Weak VEGF immunoreactivity in pre-treatment biopsies was associated with complete tumor response after neoadjuvant CRT (5-FU, cisplatin, 36 Gy) and low VEGF-A expression led to

Table 2. Drugs inhibiting VEGF signaling

Drug	Class	Function
Angiozyme (Ribozyme)	anti flt-1 ribozyme	targets VEGFR-1 expression
Bevacizumab/Avastin (Genentech)	recombinant humanized anti-VEGF antibody	neutralizing antibody against VEGF
IMC 1C11 (ImClone)	recombinant humanized receptor antibody	blocking antibody against VEGFR-2
Rapamycin (Wyeth)	mTor inhibitor	inhibits VEGF signaling pathway in target cell
SU 5416/Semaxanib (Sugen)	selektive RTK inhibitor	inhibits tyrosine kinase activity of VEGFR-2
SU 6668 (Sugen)	multitarget RTK inhibitior	inhibits tyrosine kinase activity of VEGFR-2, FGFR-1, PDGFRb
SU 11248 (Sugen)	multitarget RTK inhibitior	inhibits tyrosine kinase activity of VEGFR-2, flt-3, PDGFRb
ZD 4190 (AstraZeneca)	selektive RTK inhibitor	inhibits tyrosine kinase activity of VEGFR-2
ZD 6474 (AstraZeneca)	multitarget RTK inhibitior	inhibits tyrosine kinase activity of VEGFR-2, EGFR
ZK 222584/ PTK787 (Novartis)	selektive RTK inhibitor	inhibits tyrosine kinase activity of VEGFR-1 and VEGFR-2

RTK = Receptor tyrosine kinase.

better long-term survival after CRT and surgery. MVD showed a weak correlation with VEGF-A expression and tumor response. Comparable results were found in two recent studies by Shimada et al., where high amounts of pretreatment S-VEGF were associated with tumor progression, poor response to CRT (5-FU, cisplatin, 40 Gy) and poor survival in patients with SCC of the esophagus [38, 90]. VEGF-A expression was significantly higher in non-responders than in individuals responding to CRT. No alteration of S-VEGF levels by neo-adjuvant CRT treatment (5-FU, cisplatin, 40 Gy) was detected in the study by McDonnell et al. [39]. These authors quantified S-VEGF levels at various days before, during, and after CRT and surgery in SCC and AC patients. S-VEGF decreased below pretreatment levels 3 months after surgery reflecting the reduction of tumor mass. Nevertheless, no correlation between S-VEGF levels during CRT and tumor response to CRT was found. The authors conclude that there must be an additional source of S-VEGF which is not affected by CRT in those patients (e.g. macrophages). In conclusion, VEGF-A expression and vascularization are critical for growth and spread of EC, but also for the delivery of oxygen, drugs and radio-sensitizers. How treatment response to CRT is influenced by VEGF-A, vascular density, -permeability, and interstitial fluid pressure in EC remains controversial.

Anti-VEGF Treatment

Several studies were undertaken to show efficacy of anti-VEGF treatment in tumor models. However, until now only few authors studied options to interfere with VEGF expression in experimental esophageal carcinoma. Regarding acid-induced esophageal ulcers, Baatar et al. enhanced angiogenesis and accelerated ulcer healing in rats by local injection of plasmid cDNA encoding the recombinant human $VEGF_{165}$ isoform [17]. In contrast Gu et al. reduced $VEGF_{165}$ expression in an esophageal SCC cell line (EC109) by transfection of $VEGF_{165}$ antisense-RNA [91]. When transplanted into nude mice, the tumorigenic and angiogenic capability of the tumor cells was significantly reduced. Another group demonstrated that the anti-tumor effects of $VEGF_{165}$ antisense could be improved by placing the antisense construct under the control of a hypoxia response element (HRE). HRE then drives expression of the $VEGF_{165}$ antisense construct in hypoxic areas of the tumor where VEGF expression is maximal [92]. Regarding antiangiogenic tumor therapy in general, there are many promising new drugs tested in clinical trials worldwide (*www.cancer.gov/clinicaltrials/developments/anti-angio-table*). Some of them specifically inhibit the VEGF pathway (table 2). However, until now no published clinical data exist for selective interference with VEGF signaling in EC patients.

Conclusion

There is solid evidence that VEGF-A contributes to the aggressive characteristics of esophageal SCC and correlates with positive lymph nodes and patient's outcome. Many studies have shown a prognostic value for VEGF-A assessment in esophageal SCC and raised levels of circulating serum-VEGF have been found in many patients. In Barrett's mucosa angiogenic properties are acquired at early stages, particularly in precancerous lesions. However, VEGF-A expression patterns in AC fail to give prognostic information. Tumor vascularization and VEGF expression play a crucial role in delivery of oxygen, chemotherapeutical drugs and radiosensitizers to the tumor cells. Nevertheless, in EC patients it remains controversial how treatment response to chemo-radiotherapy is influenced by pre-treatment VEGF-A expression, vascular density, -permeability and interstitial fluid pressure. VEGF-C expression appears to be associated with lymphatic tumor invasion, lymphangiogenesis, and advanced disease in esophageal SCC and Barrett's carcinoma. To date only experimental data regarding anti-VEGF therapy in esophageal carcinoma exist. It remains to be seen whether these treatment strategies will gain clinical relevance.

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