Human Pathology (2008) **39**, 1835–1843

ELSEVIER

Human PATHOLOGY

www.elsevier.com/locate/humpath

Original contribution

High levels of vascular endothelial growth factor and its receptors (VEGFR-1, VEGFR-2, neuropilin-1) are associated with worse outcome in breast cancer☆

Sriparna Ghosh PhD[a], Catherine A.W. Sullivan MS[a], Maciej P. Zerkowski BS[b], Annette M. Molinaro PhD[c], David L. Rimm MD, PhD[d], Robert L. Camp MD, PhD[d], Gina G. Chung MD[a,*]

[a]*Section of Medical Oncology, Yale Cancer Center, Yale University School of Medicine, New Haven, CT 06520, USA*
[b]*HistoRx Inc, New Haven, CT 06511, USA*
[c]*Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, CT 06520, USA*
[d]*Department of Pathology, Yale University School of Medicine, New Haven, CT 06520, USA*

Received 31 January 2008; revised 10 June 2008; accepted 11 June 2008

Keywords:
VEGF;
Neuropilin;
Breast cancer;
Angiogenesis;
Automated image analysis

Summary Vascular endothelial growth factor has been shown to be up-regulated in breast cancers. Vascular endothelial growth factor receptors, VEGFR-1 and VEGFR-2, are the principal mediators of its effects. Together with VEGFR-1 and VEGFR-2, neuropilin-1 may act as a coreceptor for vascular endothelial growth factor. Although vascular endothelial growth factor exerts important effects on endothelial cells, VEGFRs are likely present on tumor cells as well. We used AQUA to analyze tumor-specific expression of vascular endothelial growth factor, VEGFR-1, VEGFR-2, and neuropilin-1 on a large cohort of breast cancer tissue microarray. Two-fold redundant arrays were constructed from 642 cases of primary breast adenocarcinomas. Automated image analysis with AQUA (Automated Quantitative Analysis) was then performed to determine a quantitative expression score. Scores from redundant arrays were normalized and averaged. Kaplan-Meier survival analysis showed that high levels of vascular endothelial growth factor, VEGFR-1, VEGFR-2, and neuropilin-1 were all significantly associated with survival (Miller Siegmeund corrected $P = .0020$, .0160, and .0320, respectively). In addition, vascular endothelial growth factor and neuropilin-1 retained a significant association with survival independent of other standard prognostic factors. Vascular endothelial growth factor, VEGFR-1 and -2, and neuropilin-1 are expressed to varying degrees in primary breast cancers and have prognostic significance. Further study of the functional significance of this finding is warranted as well as the prognostic value of these biomarkers in other tumor microenvironment-specific compartments (eg, vessels).

1. Introduction

Angiogenesis plays an important role in the growth and spread of cancer. Among the wide array of angiogenic factors described, vascular endothelial growth factor (VEGF; also called VEGF-A) is one of the most potent, having key

☆ G. G. Chung received support from the DOD Breast Cancer Idea Award, W81XWH-04-1-0277, Anna and Argall Hull YCC Translational Research Award, and the Susan G. Komen Breast Cancer Foundation BCTR0504220. A. M. Molinaro received support from NCI-K22CA123146.
* Corresponding author.
E-mail address: gina.chung@yale.edu (G. G. Chung).

0046-8177/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.humpath.2008.06.004

functions in the physiologic and pathophysiologic regulation of endothelial cell (EC) growth and vascular permeability [1,2]. VEGF-A also belongs to a family of related growth factors including VEGF-C, VEGF-D, and placenta growth factor that oversees modeling of the vascular system as a whole. VEGF-A will be the primary focus of this study and hereafter will be referred to as VEGF. Alternative splicing of VEGF produces 4 principal isoforms: VEGF121, VEGF165, VEGF189, and VEGF206. Two tyrosine kinase receptors, VEGFR-1 (Flt-1) and VEGFR-2 (Flk-1, KDR), identified on ECs and on bone marrow–derived elements are the principal mediators of VEGF's activities. VEGFR-2 activation leads to endothelial proliferation, survival, and permeability in part through the Raf/Mek/Erk, PI3K/Akt, and PI3K/Akt/nitric oxide pathways, respectively. The precise function of VEGFR-1 is not entirely established, and some have hypothesized it to potentially play a decoy role for VEGF. Although most studies have localized VEGF expression predominantly to tumors and stromal elements and its receptors to ECs suggesting a paracrine effect, others have demonstrated more ubiquitous expression with receptors present in the tumor as well suggesting a nonangiogenic autocrine loop [3-6].

Neuropilin-1 (NP-1) is a multifunctional non–tyrosine kinase receptor that binds to class 3 semaphorins and was originally identified for its critical role in the developing nervous system [7]. Subsequently, NP-1 was identified as a receptor for VEGF-165, the predominant isoform. In regard to VEGF-mediated signaling, NP-1 appears to function by forming coreceptor complexes with VEGFR-1 and with VEGFR-2 [8,9]. Although neuropilin expression has been described in a wide array of normal and developing tissue, its expression and regulation on vascular smooth cells and ECs (eg, up-regulation by ischemia and VEGF) suggest an important role in neoangiogensis [7]. Furthermore, NP-1 has been shown to be highly expressed in tumor-associated vasculature and in a variety of tumor cells in vitro and in situ [10,11].

Because of VEGF's potent actions on tumor-associated angiogenesis, a number of drugs targeting this growth factor and its 2 principal receptors have been developed for clinical trials, including neutralizing antibodies to VEGF or VEGFR1/2, soluble VEGF/VEGFR hybrids, multitargeted tyrosine kinase inhibitors, and direct EC toxins [12]. Although some of these agents such as the monoclonal anti-VEGF antibody bevacizumab have been successful in limited fashion in breast cancer, predictive biomarkers for these agents have not been identified. Although preclinical studies have pointed to a potential role for NP-1 in tumor growth and angiogenesis and some antitumor drugs may indirectly affect NP-1 levels, therapeutic interventions specifically targeting this receptor have not yet been extensively studied in clinical trials.

We have developed an algorithm for quantitatively determining in situ protein expression called AQUA (Automated Quantitative Analysis) [13]. AQUA is a hybrid of

Table 1 TMA patient cohort characteristics (N = 642)

	n (%)	Median (range)
Follow-up (y)		8.9 (0.19-41)
Age (y)		
<50	170 (26)	58 (24-88)
≥50	465 (73)	
Not specified	7 (1)	
Histology		
Infiltrating duct	520 (81)	
Infiltrating lobular	14 (2)	
Carcinoma (not otherwise specified)	83 (13)	
Other	25 (4)	
Tumor size (cm)		
<2	212 (33)	2.5 (0.13-14.5)
≤ 2 < 5	279 (44)	
≥5	99 (15)	
Not specified	52 (8)	
Nodal status		
Positive	317 (49)	
Negative	320 (51)	
Nuclear grade		
1	112 (17)	
2	310 (49)	
3	169 (26)	
Not Specified	51 (8)	
ER		
Positive	320 (50)	
Negative	287 (45)	
Not available	35 (5)	
PR		
Positive	298 (47)	
Negative	290 (46)	
Not available	49 (7)	
HER2		
0	368 (58)	
1	119 (19)	
2	42 (6)	
3	67 (10)	
Not specified	46 (7)	
AQUA VEGF		
High	143 (22)	
Low	402 (63)	
Unevaluable	97 (15)	
AQUA VEGFR-1		
High	187 (29)	
Low	359 (56)	
Unevaluable	96 (15)	
AQUA VEGFR-2		
High	237 (37)	
Low	315 (49)	
Unevaluable	90 (14)	
AQUA NP-1		
High	396 (62)	
Low	122 (19)	
Unevaluable	124 (19)	