Published in final edited form as:
Cancer. 2011 September 1; 117(17): 3889–3899. doi:10.1002/cncr.25935.

Prognostic Significance of Angiogenesis and Angiogenic Growth Factors in NSCLC

Ravi Salgia, M.D., Ph.D.
Department of Medicine, Section of Hematology/Oncology, The University of Chicago Medical Center, Chicago, IL

Abstract

Currently, non–small-cell lung cancer (NSCLC) is the leading cause of cancer-related death in the United States. Angiogenesis, the formation of new vasculature, is a complex and tightly regulated process that promotes metastasis and disease progression in lung cancer and other malignancies. Developmental antiangiogenic agents have shown activity in NSCLC, and bevacizumab, an antiangiogenic monoclonal antibody, is approved for the treatment of patients with advanced disease. However, predictive biomarkers are needed to guide the administration of antiangiogenic agents. It is possible that angiogenic molecules could accurately predict patient response to targeted antiangiogenic therapies, which would allow for individualized and perhaps more effective treatment. Angiogenic signaling molecules may also have value as prognostic indicators, which may be useful for the management of NSCLC. Here we provide an overview of angiogenic molecules currently being investigated as prognostic biomarkers in NSCLC and discuss their potential to guide treatment choices.

Keywords

angiogenesis; NSCLC; biomarker; antiangiogenic therapy; vascular endothelial growth factor; platelet-derived growth factor; fibroblast growth factor

Introduction

Non–small-cell lung cancer (NSCLC) is the most common type of lung carcinoma and accounts for at least 85% of all lung cancer cases in the United States.[1] Treatment options for NSCLC have included surgery, radiation, and single-agent or platinum-based doublet chemotherapy; more recently, targeted agents have been incorporated into treatment algorithms.[2] Current targeted therapy for NSCLC is limited to inhibition of the epidermal growth factor receptor (EGFR)/human epidermal growth factor receptor 1 (HER1) and vascular endothelial growth factor (VEGF). Bevacizumab (Avastin®, Genentech; South San Francisco, CA), a monoclonal antibody that targets VEGF, is the only approved antiangiogenic agent for NSCLC. Bevacizumab was approved in 2006 by the United States Food and Drug Administration as first-line treatment of patients with nonsquamous advanced, recurrent, or metastatic NSCLC in combination with carboplatin/paclitaxel (CP).[3]

Corresponding author: Ravi Salgia, MD, PhD, The University of Chicago, AMB M255, (MC 2115), 920 East 58th Street, Chicago, IL 60637, Tel: 773-702-4399, Fax: 773-834-0188, rsalgia@medicine.bsd.uchicago.edu.

Financial disclosures: Dr. Salgia receives research funding from the National Institutes of Health/National Cancer Institute. Financial support for medical and editorial assistance was provided by Boehringer Ingelheim Pharmaceuticals, Inc.

Cell surface receptors and a number of pro- and anti-angiogenic factors mediate the complex process of angiogenesis, which results in the formation of new vasculature.[4,5] There is considerable evidence associating angiogenesis with tumor growth and metastasis, and efforts are ongoing to identify angiogenic biomarkers to aid in NSCLC management.[4,6] Biomarkers are indicators of a clinical process, event, or condition and are categorized according to their specific purpose.[6] Whereas prognostic biomarkers provide information about overall patient outcome, regardless of therapy, predictive biomarkers provide information about potential therapeutic benefit.[6] Others include pharmacodynamic, toxicity, and resistance biomarkers.[6] Although angiogenic biomarkers in NSCLC are not yet validated or used in clinical practice, several measures and mediators of angiogenesis are under investigation.[7–9] Predictive/prognostic biomarkers are currently needed to guide the personalized use of antiangiogenic agents for NSCLC, currently selected by exclusion only (bevacizumab is not recommended for patients with hemorrhage or recurrent hemoptysis). Prognostic biomarkers may also be useful for weighing the benefits of continuing treatment against associated toxicities. Here we review angiogenic factors associated with NSCLC, the current understanding of their prognostic value, and their potential to predict treatment outcomes for NSCLC patients.

Angiogenic Factors as Prognostic Indicators in NSCLC

Microvessel Density

Microvessel density (MVD) is often evaluated to quantify angiogenic activity.[10,11] Intratumoral blood and lymphatic vessels can be visualized by immunohistochemical detection of specific endothelial markers such as cluster of differentiation 34 (CD340), CD31, D2-40/Podoplanin, Factor VIII for vasculature, and lymphatic vessel endothelial hyaluronan receptor-1 (LYVE-1) for lymphatic vessels. Following immunohistochemical analysis, microscopy can quantify the density of blood vessel networks.[12–15] Proteins of interest also can be analyzed for association with angiogenesis, lymphangiogenesis, or both by costaining with specific vessel markers.[16]

Several studies have demonstrated an association between MVD and patient outcomes in NSCLC,[10,15–18] although there have been conflicting reports.[11,19] In a retrospective study of 223 patients with operable NSCLC (stages IA-IIIA), higher MVD was a significant prognostic factor by univariate (hazard ratio [HR], 2.34; P=0.0001) and multivariate analysis (HR, 2.080; P=0.039).[18] A meta-analysis of published literature demonstrated that a high microvessel count within lung tumors was a poor prognostic factor for survival in patients with NSCLC.[10] However, a second meta-analysis found only weak evidence for MVD as a prognostic marker in NSCLC.[19] This variability may be due to methodological differences between studies, such as the antibody/marker used, sample selection, and counting methods.[10,11,13] Because of the lack of standardization, there has been some debate regarding the utility of MVD as a measure of angiogenesis.[13] In addition, a less invasive method is required to examine angiogenesis over time or with drug treatment.[13] Imaging biomarkers of blood flow and volume and transfer constant (K^{trans}) are of interest for monitoring response to antiangiogenic therapy.[6,20] Changes in these parameters have been visualized using MRI or CT scans within clinical trials of antiangiogenic therapy for various solid tumors, including NSCLC,[6,20] with ongoing development of other techniques for assessing tumor perfusion (eg, PET and ^{15}O-labeled water [$H_2{}^{15}O$].[21] In recent years, perfusion CT has demonstrated blood flow reductions in early-phase trials of investigational antiangiogenic agents for NSCLC; however, at present, there are many outstanding questions regarding its potential as a monitoring tool and the applicability of imaging biomarkers in guiding treatment decisions.[20]

Vascular Endothelial Growth Factor (VEGF)

VEGF-A (also known as VEGF) is an important angiogenic signaling factor, consistently associated with angiogenesis.[17,18,22–24] Human VEGF exists in 6 known isoforms ($VEGF_{121}$, $VEGF_{145}$, $VEGF_{165}$, $VEGF_{183}$, $VEGF_{189}$, and $VEGF_{206}$) due to alternative splicing.[25] Although all 3 secreted isoforms ($VEGF_{121}$, $VEGF_{145}$ and $VEGF_{165}$) induce angiogenesis, $VEGF_{165}$ is the predominant isoform, and is overexpressed in a variety of tumors.[25,26] Other VEGF family members, including placenta growth factor (PlGF) -1 and -2, VEGF-B, VEGF-C, and VEGF-D, are also involved in angiogenic processes.[23,26] Early studies identified VEGF as a significant prognostic factor in NSCLC by immunohistochemical analysis, reverse transcriptase polymerase chain reaction (RT-PCR), or immunoassay.[17,18,27] Recent studies of the prognostic value of VEGF in NSCLC have generally supported these earlier results, and some have also examined other VEGF family members (Table 1). As with MVD, it is likely that variations in laboratory technique and study design (ie, patient characteristics) have contributed to the variable results from studies of VEGF. However, of all the molecules examined as biomarkers in NSCLC, VEGF has most consistently been correlated with patient outcomes.

Other forms of VEGF, resulting from alternative splicing and single nucleotide polymorphisms (SNPs), have been evaluated for prognostic significance in NSCLC.[7,28,29] Variations in genomic sequence among individuals, called SNPs, may cause changes in levels, function, and/or activity of transcribed proteins.[7] Much of the analysis in NSCLC has focused on SNPs as potential risk factors of developing disease, but several SNPs have been associated with VEGF expression and angiogenesis.[30] Investigation of alternative VEGF isoforms as prognostic factors in NSCLC has been limited but results have been fairly consistent. Among 57 NSCLC samples analyzed by RT-PCR, a high $VEGF_{189}$ expression ratio was correlated with shorter survival (median, 18.0 vs. 40 months; P=0.0001) and earlier postoperative disease recurrence (mean time to recurrence, 5 vs. 26 months; P=0.0086).[28] A recent RT-PCR analysis of 130 NSCLC specimens found highly significant (all comparisons P<0.0001) coexpression among the isoforms analyzed (VEGF, $VEGF_{121}$, $VEGF_{165}$, and $VEGF_{189}$) and identified $VEGF_{189}$ as an independent prognostic indicator by multivariate analysis (P=0.025).[29]

VEGF has also been investigated in NSCLC as a predictive biomarker for response to antiangiogenic therapy. In a prospective study of a randomized phase II/III trial evaluating CP alone or with bevacizumab (BCP) in 898 patients with advanced NSCLC, patients with high plasma VEGF levels had an increased probability of response with BCP versus CP (33% vs 7%, P=0.01).[31] However, VEGF levels were not predictive of the survival benefit observed in the BCP arm.[31] The authors postulated that the VEGF-predicted response improvement may not have been related to the survival benefit of bevacizumab.[31] It was also noted that, because binding of bevacizumab may increase the half-life of VEGF and limit its detectability, analysis of VEGF levels by immunoassay in patients treated with bevacizumab may result in misleading measurements.[31] In the preliminary results of a pharmacogenetic subanalysis of this same study, VEGF polymorphism (G-634C) was 1 of the significant predictors of overall survival (P<0.05).[32] More recently, preliminary results of a similar pharmacogenetic analysis of plasma samples from 88 patients who participated in a randomized phase II trial of sorafenib (Nexavar®, Bayer; Leverkusen, Germany) in advanced NSCLC suggest that germline VEGF polymorphisms may impact the disease control (DC) rate and progression-free survival.[33]

VEGF has also been examined in the context of treatment with vandetanib (Zactima™, AstraZeneca; Wilmington, DE), a small molecule inhibitor of VEGF signaling, EGFR signaling to a lesser extent, and rearranged during transfection (RET) tyrosine kinases.[34,35] A summary was recently published of 3 studies that evaluated VEGF as a predictive marker

of response to vandetanib in patients with NSCLC, 1 of which was terminated early.[34] In the other 2 studies in chemotherapy-pretreated patients, low baseline plasma VEGF levels was associated with superior progression-free survival with vandetanib versus gefitinib (Iressa®, AstraZeneca; Wilmington, DE) (HR, 0.55; 95% confidence interval [CI], 0.35–0.86; *P*=0.01) and with docetaxel plus vandetanib 100 mg versus docetaxel plus placebo (HR, 0.25; 95% CI, 0.09–0.68; *P*=0.01).[34] Although several phase III trials of vandetanib have not demonstrated substantial clinical benefit in patients with NSCLC,[36–39] these results suggest that VEGF levels may have predictive value in the management of lung cancer. More recently, Hanrahan and colleagues analyzed whether 35 different cytokines and angiogenic factors (CAFs), including VEGF, were affected with vandetanib in 123 patients with NSCLC enrolled in a randomized phase II trial.[40] Patients received vandetanib monotherapy, CP, or CP plus vandetanib, and VEGF levels were analyzed at baseline and on Days 8, 22, and 43.[40] In the vandetanib monotherapy arm, VEGF levels were significantly elevated at Day 43 (*P*=0.048).[40] VEGF elevations have also been observed preclinically in several tumor types after sunitinib (SUTEN®, Pfizer; New London, CT), an inhibitor of the VEGF and platelet-derived growth factor (PDGF) pathways.[41,42]

Vascular Endothelial Growth Factor Receptors (VEGFRs)

The VEGF family of ligands activates 3 receptor tyrosine kinases (RTKs): VEGFR-1/fms-like tyrosine kinase 1 (Flt1), VEGFR-2/kinase insert domain receptor (KDR), and VEGFR-3/Flt4.[23,26,43] VEGFR-1, expressed in the vasculature, can act as a negative regulator of angiogenesis.[26] VEGFR-2 plays a primary role in vasculogenesis and may stimulate angiogenesis.[26] Like other VEGFRs, VEGFR-3 is essential for development, where it functions in cardiovascular development and vascular remodeling.[26,43] During adulthood, VEGFR-3 is primarily associated with lymphangiogenesis.[26,44] Both VEGFR-2 and -3, but particularly VEGFR-3, have been implicated in lymphatic metastasis.[44,45] The variability in the prognostic relevance of VEGFR expression is illustrated by the results summarized in Table 2. VEGFR-3 has been most commonly reported as an indicator for poor clinical outcomes in NSCLC.[46–48] Similar results have been demonstrated with the other 2 VEGFRs,[47–49] but other studies have failed to show a significant association with clinical outcomes in NSCLC.[49–51] VEGFR-2 was analyzed among the 35 CAFs examined in the study by Hanrahan and colleagues; notably, VEGFR-2 serum levels were significantly lowered 8 days after treatment with vandetanib among all treatment arms (*P*=0.001) and at Day 43 in the vandetanib monotherapy arm (*P*<0.001).[40] Additional parameters that have been associated with prognosis of NSCLC include VEGFR-3-positive endothelial cell density[52] and the ratio of *VEGF* to *VEGFR-1* expression by RT-PCR.[53]

Recently, VEGF and VEGFR-2 were investigated as predictive biomarkers in patients with advanced NSCLC as part of a large prospective clinical trial program, BATTLE (Biomarker-integrated Approaches of Targeted Therapy for Lung Cancer Elimination).[54] Patients were heavily pretreated (at least 2 prior regimens) and enrolled in an umbrella study where core biopsy samples were screened for 11 biomarkers, including VEGF and VEGFR-2 expression. Molecular characteristics of the EGFR, Ras/Raf, and cyclin D1/retinoid X receptor (RXR) pathways were also examined. Based on the biomarker analysis, patients were assigned to receive erlotinib, sorafenib, vandetanib, or erlotinib/bexarotene.[54–56] Two hundred-fifty five patients were randomized, and the overall 8-week DC rate (primary endpoint) was 46%. Among patients in the VEGF marker group treated with sorafenib (n=39), the 8-week DC rate was 64%,[54] with a similarly high DC rate of 61% (11/18) subsequently reported for sorafenib in *Kirsten rat sarcoma* (*KRAS*) mutation-positive patients but not in patients with *EGFR* mutation-positive tumors (23% [3/13]) or EGFR high-polysomy (27% [3/11]).[56] In addition, high VEGFR-2 expression significantly correlated with improved outcome with vandetanib treatment.[54] This study represents a

major step toward molecularly based personalized medicine in NSCLC. Additional BATTLE studies are planned in chemorefractory patients (BATTLE-2) and in patients with metastatic disease (BATTLE-3).[54]

PDGF and PDGFRs

The PDGF family of ligands is composed of 5 different dimeric isoforms (PDGF-AA, -BB, -CC, -DD, and -AB) that bind and activate 2 receptor tyrosine kinases, PDGFR-α and PDGFR-β.[57,58] PDGF signaling has been found to play a crucial role in organogenesis during embryonic development, and is implicated in a variety of conditions including cardiovascular and fibrotic diseases.[59] Initially discovered because of its effects on cellular proliferation,[57] PDGF signaling has since been identified as a promoter of angiogenesis and metastasis through recruitment of stroma (mesenchymal cells and blood vessels) and fibroblasts.[59,60] For example, it is thought that paracrine PDGF pathway signaling promotes pericyte recruitment to tumor blood vessels, which may lead to stabilization of vasculature and promote tumor growth.[59–61] Because of its role in angiogenesis, it has been suggested that PDGF signaling may also play a role in development of resistance to antiangiogenic therapies that target the VEGF pathway.[62] A preclinical study demonstrated that upregulated PDGF-C in tumor-associated fibroblasts was associated with resistance to anti-VEGF treatment in lymphoma cell lines.[62] Interestingly, the source of the redundant angiogenic signaling was a component of the stromal cells rather than the tumor cell population.[62] These results suggest that in the context of antiangiogenic therapy, tumor stromal cells may significantly influence efficacy. Consequently, a more complete understanding of the crosstalk between these tissues is necessary.

Though PDGF ligands and receptors have been evaluated as prognostic factors in a number of malignancies,[63–65] studies in NSCLC have begun only recently. In a TMA study of tumor samples from 55 patients with NSCLC who received adjuvant postoperative radiotherapy, univariate analysis demonstrated that high PDGF levels correlated with poor survival (P=0.002),[48] with high tumor PDGF expression independently associated with shorter DSS by multivariate analysis (HR, 5.42; 95% CI, 1.93–15.2; P=0.002).[48] In another TMA study of samples from 335 resected patients with stage I-IIIA NSCLC, high tumor cell expression of PDGF-B (P=0.001), PDGF-C (P=0.01), and PDGFR-α (P=0.026) were negative prognostic indicators for DSS by univariate analysis, while multivariate analysis identified high tumor expression of PDGF-B (P=0.001) and PDGFR-α (P=0.047) as independent negative prognostic indicators.[8] High stromal expression of PDGF-A (P=0.009), PDGF-B (P=0.04), PDGF-D (P=0.019), and PDGFR-α (P=0.019) were identified as positive prognostic indicators for DSS by univariate analysis; high stromal PDGF-A expression (P=0.001) was an independent positive indicator for survival by multivariate analysis.[8] Although results are suggestive of a prognostic role for PDGF in NSCLC, additional studies will be necessary to validate candidate members of the pathway.

Fibroblast Growth Factor (FGF)

The FGF family of ligands has 22 members that exert their cellular functions by binding and activating 4 FGF receptor (FGFR1-4) family members.[66] Although there are only 4 receptors, alternative splicing events can create receptor diversity by increasing selectivity of binding to FGF ligands.[66] The complex cellular signaling network created by interactions of FGFs and FGFRs impacts a variety of normal and pathological processes including chemotaxis, tissue development, angiogenesis, inflammation, and tumorigenesis.[66] FGF2 (basic FGF or bFGF) is expressed by many tumor types and plays an important role in tumor cell proliferation and angiogenesis.[66,67] FGF2 has been shown to have mitogenic and migratory effects on endothelial cells, fibroblasts, and smooth muscle cells; however, because mice deficient in FGF2 retain normal vascularization, the precise role of FGF2 in

angiogenesis is unclear.[67,68] Similar to the PDGF pathway, FGF signaling may also play a role in resistance to VEGF inhibition. In a preclinical study, after a period of VEGFR-2 inhibition (phase 1), concomitant inhibition of FGF and VEGF signaling (phase 2) caused a greater decrease in angiogenesis than VEGFR2 blockade alone (phase 2) in late-stage pancreatic islet tumors.[69] Just as FGF signaling may contribute to angiogenesis under conditions of VEGF pathway inhibition, it is possible that other compensatory growth signals may allow for normal vascularization in the absence of FGF.[68,69] Indeed, in the preclinical study described, tumor burden and angiogenic measures were slightly higher in phase 2 even with combined VEGF/FGF inhibition, than with maximal response to initial VEGF inhibition in phase 1, suggesting that other factors stimulated angiogenesis and tumor growth in this system.[69]

Members of the FGF pathway have been investigated as prognostic factors in multiple malignancies, although evaluation in NSCLC has been more recent and has focused on bFGF.[25,70] In a TMA study of samples from 335 patients with NSCLC, high tumor cell FGF2 expression was a negative prognostic factor for DSS (P=0.015) and identified as an independent negative prognostic factor by multivariate analysis (P=0.038).[71] In addition, coexpression of high levels of FGF2/VEGFR-3 (P<0.001), FGF2/PDGF-B (P=0.002), FGFR-1/VEGFR-3 (P=0.001), and FGFR-1/PDGF-B (P=0.002) in tumor cells were negative prognostic indicators for DSS. Coexpression of high levels of FGF2/VEGFR-3 (P<0.001) and FGFR-1/VEGFR-3 (P=0.001) were also correlated with lymph node metastasis.[71] By univariate analysis, high stromal FGF2 expression was a positive prognostic factor for DSS (P=0.024), and by multivariate analysis, it was identified as an independent positive prognostic factor (P=0.015).[71] Another study of 71 patients with NSCLC reported that high bFGF levels were associated with poor survival (P=0.0059), as was high bFGF/VEGF expression (P<0.0001).[72] By multivariate analysis, both bFGF (P=0.0242) and VEGF (P=0.0428) were independent prognostic indicators for survival.[72] In another TMA study, high stromal bFGF expression was correlated with improved survival (P=0.017) by univariate analysis, and independently associated with increased DSS by multivariate analysis (HR, 0.077; 95% CI, 0.015–0.40; P<0.001).[48] However, a recent retrospective analysis of samples from patients with NSCLC did not find bFGF to correlate with patient outcomes.[73] A literature analysis on the prognostic value of circulating bFGF levels in NSCLC reported inconsistent results as well.[25]

MicroRNAs

Small noncoding RNAs, called microRNAs (miRNAs), are newly discovered regulators of angiogenesis and may prove useful for prognostic efforts in NSCLC.[74,75] MiRNAs negatively affect protein translation at the posttranscriptional level and may affect many pathways relevant for tumor progression and metastasis.[75] An estimated 700 miRNAs have been identified in the human genome thus far,[75] and several reports indicate that expression of a specific miRNA signature is associated with lung cancer.[76–79] In the study by Yanaihara and colleagues, 43 of 147 miRNAs examined were expressed at significantly different levels in cancerous versus matched normal tissue.[77] By univariate analysis, high expression of 5 miRNAs and low expression of 3 miRNAs were associated with a worse prognosis in patients with adenocarcinoma; high expression of *miR-155* (P=0.006) and low expression of *let-7a-2* (P=0.033) were associated with poor survival and multivariate analysis identified *miR-155* expression as an independent unfavorable prognostic factor (HR, 3.03; 95% CI, 1.13–8.14; P=0.027).[77] In another study, high expression of *miR-155* (HR, 2.3; 95% CI, 1.0–5.6; P=0.06) and *miR-146b* (HR, 2.7; 95% CI, 1.4–5.7; P=0.0035) were associated with worse overall survival in squamous cell lung carcinoma.[79] Yu and colleagues identified a 5-miRNA signature (consisting of *let-7a*, *miR-221*, *miR-137*, *miR-372*, and *miR-182*), from 157 miRNAs evaluated, as an independent prognostic factor

for survival in lung cancer (HR, 2.81; 95% CI, 1.13–7.01; *P*=0.026).[78] One of these, *miR-221*, has been reported to play a role in angiogenesis in the tumor microenvironment, while others are involved in proliferation and anchorage-independent growth.[78] It is well known that 1 miRNA may affect many targets, and thus, many processes.[78] As a result, it is challenging to determine how differential expression of a miRNA specifically affects tumor cell function; in fact, 1 miRNA could potentially function as a tumor suppressor and promoter.[78]

Interleukins

Interleukins are secreted chemokines involved in a wide range of signaling processes, including inflammation, tumor progression, and angiogenesis.[80–82] In a study of 60 patients with NSCLC and a history of smoking, plasma levels of interleukin-8 (IL-8), an angiogenic chemokine, were reported to be significantly higher in stage IV (median 131.4 pg/ml, CI, 135.01]) versus stage III disease (median 61.7 pg/ml, CI, 39.7; *P*=0.012).[83] While not identified as an independent prognostic indicator in the study, baseline serum IL-8 levels were elevated in patients with NSCLC with respect to control patients (*P*<0.0001).[83] In another study of patients with NSCLC (N=58), 38 of whom received surgery and 20 of whom received postoperative chemotherapy and/or radiation, IL-8 mRNA levels via quantitative RT-PCR and IL-8 protein was analyzed by immunohistochemistry.[84] IL-8 expression was significantly elevated in tumor samples versus matched normal lung tissue (*P*=0.012), and patients with tumors exhibiting high versus low IL-8 expression were more likely to have advanced disease (Stage IIIA or IIIB; *P*=0.03), distant lymph node metastasis (*P*=0.02), a high tumor microvessel count (*P*=0.00003), shorter survival (<26 months; *P*<0.00001), and earlier relapse (<16 months; *P*<0.00001).[84] IL-8 mRNA expression was also identified as a prognostic factor for survival (*P*<0.0001) and prediction of recurrence (*P*=0.0001) by multivariate analysis.[84] Another study tested for an association between IL-8 and NSCLC prognosis without success.[73] IL-17, an inflammatory cytokine, has been shown to promote angiogenesis and is frequently detected in many inflammation-associated cancers, including NSCLC.[85] In an analysis of tissue from 52 patients, IL-17 was identified as an independent prognostic factor for disease-free survival (HR, 3.036; 95% CI, 1.345–6.852; *P*=0.007) and overall survival (HR, 2.869; 95% CI, 1.274–6.460; *P*=0.011).[85] IL-17-positive tumors also had a significantly higher lymphatic vessel density than IL-17-negative tumors (*P*=0.008), suggesting a possible role in lymphangiogenesis as well as angiogenesis.[85]

Unlike the proangiogenic IL-8 and IL-17, IL-12 has been characterized as a strongly antiangiogenic cytokine.[86] The utility of pretreatment circulating IL-12 levels as a predictive biomarker for antiangiogenic therapy was recently described for the first time, with an exploratory analysis of a phase II trial of pazopanib (GlaxoSmithKline; London, UK) as neoadjuvant monotherapy for early-stage NSCLC demonstrating that baseline plasma levels of IL-12 were most strongly correlated with tumor size reduction among 31 CAFs (*P*=0.00065).[87]

Several interleukins were included in the panel of 35 CAFs in the aforementioned study by Hanrahan and colleagues.[40] In the vandetanib monotherapy arm, IL-8 levels were increased at Day 8 (*P*=0.041) and IL-17 was increased at Day 43 (*P*=0.045). Significant decreases in IL-12 were observed at Day 8 in the CPV (*P*<0.001) and CP arms (*P*<0.001). In the CPV arm, significant correlations were noted for elevations in IL-8 at Day 8 and poorer progression-free survival (HR, 1.48; 95% CI, 1.02–2.16), as well as elevated IL-12 at Day 8 and improved progression-free survival (HR, 0.61; 95% CI, 0.40–0.94).[40]

Other Factors

Cyclo-oxygenase-2 (COX-2), an enzyme well known for its role in inflammation and pain,[88] has also been implicated in angiogenic processes.[89] Results of a study of 232 NSCLC patients who underwent complete resection indicated that COX-2 expression measured by immunohistochemistry correlated significantly with prognosis (HR, 2.280; 95% CI, 1.255–4.143; P=0.0068) by univariate analysis, but not by multivariate analysis.[90] In another study of 70 patients with NSCLC, samples with high COX-2 mRNA levels measured by RT-PCR had a significantly higher microvessel count than samples with low COX-2 levels (P<0.001).[91] Additionally, patients with high COX-2 levels had significantly shorter survival time (mean survival, 15 vs. 40 months; P<0.0001) and faster relapse (mean time to relapse, 5.0 vs. 34.0 months; P<0.0001) than patients with low COX-2 levels.[91] Another study reported no correlation between COX-2 and prognosis.[92]

Cadherins, transmembrane glycoproteins that regulate cell-cell adhesion, are among the adhesion molecules associated with tumor angiogenesis and poor survival specifically in the NSCLC population.[93] In a retrospective review of 150 patients with NSCLC, expression of E-cadherin did not correlate with vascularity; however, hypervascularity was significantly higher in tumors positive for N-cadherin (67.4% vs. 49.0%; P=0.0373).[93] The impact of N-cadherin-positivity on 5-year survival was limited to undifferentiated large-cell carcinoma (0% vs. 55.6%; P=0.0013). E-cadherin-negativity was associated with lower survival in the overall population (45.4% vs. 64.4%; P=0.0146) and was a significant predictor for poor survival by multivariate analysis (HR, 1.736; P=0.0339). Most recently, in a 62-patient study to evaluate serum levels of soluble E-cadherin, intracellular adhesion molecule-1 (ICAM-1)/CD54, and E-selectin/CD62E as biomarkers in lung cancer, levels of all 3 adhesion molecules were significantly elevated in both small cell and NSCLC versus the healthy controls (P<0.001), with a correlation between only E-cadherin levels and the presence of distant metastases.[94] Conversely, in a previously reported prospective study of the prognostic value of E-selectin and ICAM-1 in 57 chemotherapy-treated patients with advanced NSCLC, serum E-selectin levels were 1 of only 2 independent prognosticators for survival by multivariate analysis (P=0.002), the other being weight loss.[95] In the biomarker analysis of the E4599 phase II/III trial of bevacizumab for advanced NSCLC, low baseline ICAM levels were significantly associated with improvements in both response rate (P=0.03) and survival (P=0.00005) in the CP and BCP arms, supporting further evaluation of adhesion molecules as both predictive and prognostic biomarkers.[31]

Inhibitors of angiogenesis, such as collagen XVIII (precursor of endostatin) and angiostatin, have also been investigated as potential prognostic factors in NSCLC. In a study of tissue from 221 patients with NSCLC, collagen XVIII was identified as a significant prognostic indicator by immunohistochemical analysis (P=0.0015).[96] These results were supported by an immunohistochemical study of 94 patients with NSCLC that demonstrated expression of collagen XVIII was an independent negative prognostic factor; the multivariate analysis included samples that were strongly positive versus negative (HR, 3.605; 95% CI, 1.305–9.958; P=0.0134) and weakly positive versus negative (HR, 4.612; 95% CI, 1.361–15.633; P=0.0141).[97] More recently, however, endostatin was not validated as a prognostic factor in NSCLC from a panel of investigated molecules.[98]

Conclusions and Future Directions

At this time, there are no reliable prognostic or predictive angiogenic markers in the NSCLC population. Identification and validation of predictive biomarkers will be necessary to personalize antiangiogenic treatment for NSCLC patients. Several potential angiogenic prognostic factors are already under investigation because of their potential clinical utility. As more proteins and molecules relevant for angiogenic processes are discovered and

characterized, perhaps new candidate biomarkers will become available for evaluation in NSCLC. Known mediators and inhibitors of angiogenic processes are currently under investigation as biomarkers in NSCLC. It is possible that some of these factors could be validated as prognostic factors, or as predictive factors, if their measures correspond with treatment outcome. Large prospective clinical trials, such as BATTLE, are needed to evaluate candidate biomarkers, and additional confirmatory studies will be necessary for validation. It is also likely that advances in sensitivity, specificity, and standardization of assays will be necessary before these tools may be streamlined for routine clinical use.[99]

Acknowledgments

Writing and editorial assistance was provided by Staci Deaton, PhD of MedErgy, which was contracted by BIPI for these services. The author meets criteria for authorship as recommended by the International Committee of Medical Journal Editors (ICMJE), was fully responsible for all content and editorial decisions, and was involved at all stages of manuscript development. The author received no compensation related to the development of the manuscript.

References

1. American Cancer Society. Cancer Facts & Figures, 2009. Atlanta, GA: American Cancer Society, Inc; 2009.
2. National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology™. Non-Small Cell Lung Cancer. [accessed Apr. 8, 2010]. V.2.2010. Available from URL: http://www.nccn.org/professionals/physician_gls/PDF/nscl.pdf
3. Avastin. Avastin® (bevacizumab) [package insert]. South San Francisco, CA; Genentech, Inc: July. 2009 p. 2009
4. Carmeliet P, Jain RK. Angiogenesis in cancer and other diseases. Nature. 2000; 407:249–257. [PubMed: 11001068]
5. Folkman J, Klagsbrun M. Vascular physiology. A family of angiogenic peptides. Nature. 1987; 329:671–672. [PubMed: 2444887]
6. Jain RK, Duda DG, Willett CG, et al. Biomarkers of response and resistance to antiangiogenic therapy. Nat Rev Clin Oncol. 2009; 6:327–338. [PubMed: 19483739]
7. Jain L, Vargo CA, Danesi R, et al. The role of vascular endothelial growth factor SNPs as predictive and prognostic markers for major solid tumors. Mol Cancer Ther. 2009; 8:2496–2508. [PubMed: 19755511]
8. Donnem T, Al-Saad S, Al-Shibli K, Andersen S, Busund LT, Bremnes RM. Prognostic impact of platelet-derived growth factors in non-small cell lung cancer tumor and stromal cells. J Thorac Oncol. 2008; 3:963–970. [PubMed: 18758297]
9. Oldenhuis CN, Oosting SF, Gietema JA, de Vries EG. Prognostic versus predictive value of biomarkers in oncology. Eur J Cancer. 2008; 44:946–953. [PubMed: 18396036]
10. Meert AP, Paesmans M, Martin B, et al. The role of microvessel density on the survival of patients with lung cancer: a systematic review of the literature with meta-analysis. Br J Cancer. 2002; 87:694–701. [PubMed: 12232748]
11. Kreuter M, Kropff M, Fischaleck A, et al. Prognostic relevance of angiogenesis in stage III NSCLC receiving multimodality treatment. Eur Respir J. 2009; 33:1383–1388. [PubMed: 19213790]
12. Choi WW, Lewis MM, Lawson D, et al. Angiogenic and lymphangiogenic microvessel density in breast carcinoma: correlation with clinicopathologic parameters and VEGF-family gene expression. Mod Pathol. 2005; 18:143–152. [PubMed: 15297858]
13. Pathak AP, Hochfeld WE, Goodman SL, Pepper MS. Circulating and imaging markers for angiogenesis. Angiogenesis. 2008; 11:321–335. [PubMed: 18925424]
14. Renyi-Vamos F, Tovari J, Fillinger J, et al. Lymphangiogenesis correlates with lymph node metastasis, prognosis, and angiogenic phenotype in human non-small cell lung cancer. Clin Cancer Res. 2005; 11:7344–7353. [PubMed: 16243806]

15. Mineo TC, Ambrogi V, Baldi A, et al. Prognostic impact of VEGF, CD31, CD34, and CD105 expression and tumour vessel invasion after radical surgery for IB-IIA non-small cell lung cancer. J Clin Pathol. 2004; 57:591–597. [PubMed: 15166262]

16. Kadota K, Huang CL, Liu D, et al. The clinical significance of lymphangiogenesis and angiogenesis in non-small cell lung cancer patients. Eur J Cancer. 2008; 44:1057–1067. [PubMed: 18396396]

17. Han H, Silverman JF, Santucci TS, et al. Vascular endothelial growth factor expression in stage I non-small cell lung cancer correlates with neoangiogenesis and a poor prognosis. Ann Surg Oncol. 2001; 8:72–79. [PubMed: 11206229]

18. O'Byrne KJ, Koukourakis MI, Giatromanolaki A, et al. Vascular endothelial growth factor, platelet-derived endothelial cell growth factor and angiogenesis in non-small-cell lung cancer. Br J Cancer. 2000; 82:1427–1432. [PubMed: 10780522]

19. Trivella M, Pezzella F, Pastorino U, Harris AL, Altman DG. Microvessel density as a prognostic factor in non-small-cell lung carcinoma: a meta-analysis of individual patient data. Lancet Oncol. 2007; 8:488–499. [PubMed: 17513172]

20. Ng QS, Goh V. Angiogenesis in non-small cell lung cancer: imaging with perfusion computed tomography. J Thorac Imaging. 2010; 25:142–150. [PubMed: 20463533]

21. de Langen AJ, Lubberink M, Boellaard R, et al. Reproducibility of tumor perfusion measurements using 15O-labeled water and PET. J Nucl Med. 2008; 49:1763–1768. [PubMed: 18927324]

22. Folkman J, Shing Y. Angiogenesis. J Biol Chem. 1992; 267:10931–10934. [PubMed: 1375931]

23. Lohela M, Bry M, Tammela T, Alitalo K. VEGFs and receptors involved in angiogenesis versus lymphangiogenesis. Curr Opin Cell Biol. 2009; 21:154–165. [PubMed: 19230644]

24. Nakashima T, Huang C-L, Liu D, et al. Expression of vascular endothelial growth factor-A and vascular endothelial growth factor-C as prognostic factors for non-small cell lung cancer. Med Sci Monit. 2004; 10:BR157–BR165. [PubMed: 15173661]

25. Bremnes RM, Camps C, Sirera R. Angiogenesis in non-small cell lung cancer: the prognostic impact of neoangiogenesis and the cytokines VEGF and bFGF in tumours and blood. Lung Cancer. 2006; 51:143–158. [PubMed: 16360975]

26. Hicklin DJ, Ellis LM. Role of the vascular endothelial growth factor pathway in tumor growth and angiogenesis. J Clin Oncol. 2005; 23:1011–27. [PubMed: 15585754]

27. Yuan A, Yu C-J, Chen W-J, et al. Correlation of total VEGF mRNA and protein expression with histologic type, tumor angiogenesis, patient survival and timing of relapse in non-small-cell lung cancer. Int J Cancer. 2000; 89:475–483. [PubMed: 11102890]

28. Yuan A, Yu C-J, Kuo S-H, et al. Vascular endothelial growth factor 189 mRNA isoform expression specifically correlates with tumor angiogenesis, patient survival, and postoperative relapse in non-small-cell lung cancer. J Clin Oncol. 2001; 19:432–441. [PubMed: 11208836]

29. Matsuyama M, Chijiwa T, Inoue Y, et al. Alternative splicing variant of vascular endothelial growth factor-A is a critical prognostic factor in non-small cell lung cancer. Oncol Rep. 2009; 22:1407–1413. [PubMed: 19885594]

30. Koukourakis MI, Papazoglou D, Giatromanolaki A, Bougioukas G, Maltezos E, Sivridis E. VEGF gene sequence variation defines VEGF gene expression status and angiogenic activity in non-small cell lung cancer. Lung Cancer. 2004; 46:293–298. [PubMed: 15541813]

31. Dowlati A, Gray R, Sandler AB, Schiller JH, Johnson DH. Cell adhesion molecules, vascular endothelial growth factor, and basic fibroblast growth factor in patients with non-small cell lung cancer treated with chemotherapy with or without bevacizumab-an Eastern Cooperative Oncology Group Study. Clin Cancer Res. 2008; 14:1407–1412. [PubMed: 18316562]

32. Zhang W, Dahlberg SE, Yand D, et al. Genetic variants in angiogenesis pathway associated with clinical outcome in NSCLC patients (pts) treated with bevacizumab in combination with carboplatin and paclitaxel: subset pharmacogenetic analysis of ECOG 4599. J Clin Oncol. 2009; 27 Abstract 8032.

33. Zhang W, Lee J, Schiller JH, Carbone DP, Chung CH, Lenz H. Use of germline polymorphisms in VEGF to predict tumor response and progression-free survival in non-small cell lung cancer (NSCLC) patients treated with sorafenib: Subset pharmacogenetic analysis of Eastern Cooperative Oncology Group (ECOG) trial E2501. J Clin Oncol. 2010; 28 Abstract 7607.

34. Hanrahan EO, Ryan AJ, Mann H, et al. Baseline vascular endothelial growth factor concentration as a potential predictive marker of benefit from vandetanib in non-small cell lung cancer. Clin Cancer Res. 2009; 15:3600–3609. [PubMed: 19447868]
35. Morabito A, Piccirillo MC, Falasconi F, et al. Vandetanib (ZD6474), a dual inhibitor of vascular endothelial growth factor receptor (VEGFR) and epidermal growth factor receptor (EGFR) tyrosine kinases: current status and future directions. Oncologist. 2009; 14:378–390. [PubMed: 19349511]
36. Herbst RS, Sun Y, Korfee S, et al. Vandetanib plus docetaxel versus docetaxel as second-line treatment for patients with advance non-small cell lung cancer (NSCLC): a randomized, double-blind phase III trial (ZODIAC). J Clin Oncol. 2009; 27:807s. Abstract CRA8003.
37. De Boer R, Arrieta O, Gottfried M, et al. Vandetanib plus pemetrexed versus pemetrexed as second-line therapy in patients with advanced non-small cell lung cancer (NSCLC): a randomized, double-blind phase III trial (ZEAL). J Clin Oncol. 2009; 27:409s. Abstract 8010.
38. Natale RB, Thongprasert S, Greco FA, et al. Vandetanib versus erlotinib in patients with advanced non-small cell lung cancer (NSCLC) after failure of at least one prior cytotoxic chemotherapy: a randomized, double-blind phase III trial (ZEST). J Clin Oncol. 2009; 27:409s. Abstract 8009.
39. Lee J, Hirsh V, Park K, et al. Vandetanib versus placebo in patients with advanced non-small cell lung cancer (NSCLC) after prior therapy with an EGFR tyrosine kinase inhibitor (TKI): a randomized, double-blind phase III trial (ZEPHYR). J Clin Oncol. 2010; 28:7525.
40. Hanrahan EO, Lin HY, Kim ES, et al. Distinct patterns of cytokine and angiogenic factor modulation and markers of benefit for vandetanib and/or chemotherapy in patients with non-small-cell lung cancer. J Clin Oncol. 2010; 28:193–201. [PubMed: 19949019]
41. Faivre S, Delbaldo C, Vera K, et al. Safety, pharmacokinetic, and antitumor activity of SU11248, a novel oral multitarget tyrosine kinase inhibitor, in patients with cancer. J Clin Oncol. 2006; 24:25–35. [PubMed: 16314617]
42. Motzer RJ, Michaelson MD, Redman BG, et al. Activity of SU11248, a multitargeted inhibitor of vascular endothelial growth factor receptor and platelet-derived growth factor receptor, in patients with metastatic renal cell carcinoma. J Clin Oncol. 2006; 24:16–24. [PubMed: 16330672]
43. Ellis LM, Hicklin DJ. VEGF-targeted therapy: mechanisms of anti-tumour activity. Nat Rev Cancer. 2008; 8:579–591. [PubMed: 18596824]
44. van Iterson V, Leidenius M, von Smitten K, Bono P, Heikkila P. VEGF-D in association with VEGFR-3 promotes nodal metastasis in human invasive lobular breast cancer. Am J Clin Pathol. 2007; 128:759–766. [PubMed: 17951197]
45. Goldman J, Rutkowski JM, Shields JD, et al. Cooperative and redundant roles of VEGFR-2 and VEGFR-3 signaling in adult lymphangiogenesis. FASEB J. 2007; 21:1003–1012. [PubMed: 17210781]
46. Arinaga M, Noguchi T, Takeno S, Chujo M, Miura T, Uchida Y. Clinical significance of vascular endothelial growth factor C and vascular endothelial growth factor receptor 3 in patients with nonsmall cell lung carcinoma. Cancer. 2003; 97:457–464. [PubMed: 12518370]
47. Donnem T, Al-Saad S, Al-Shibli K, et al. Inverse prognostic impact of angiogenic marker expression in tumor cells versus stromal cells in non small cell lung cancer. Clin Cancer Res. 2007; 13:6649–6657. [PubMed: 18006765]
48. Andersen S, Donnem T, Al-Saad S, Al-Shibli K, Busund LT, Bremnes RM. Angiogenic markers show high prognostic impact on survival in marginally operable non-small cell lung cancer patients treated with adjuvant radiotherapy. J Thorac Oncol. 2009; 4:463–471. [PubMed: 19204576]
49. Seto T, Higashiyama M, Funai H, et al. Prognostic value of expression of vascular endothelial growth factor and its flt-1 and KDR receptors in stage I non-small-cell lung cancer. Lung Cancer. 2006; 53:91–96. [PubMed: 16697074]
50. Decaussin M, Sartelet H, Robert C, et al. Expression of vascular endothelial growth factor (VEGF) and its two receptors (VEGF-R1-Flt1 and VEGF-R2-Flk1/KDR) in non-small cell lung carcinomas (NSCLCs): correlation with angiogenesis and survival. J Pathol. 1999; 188:369–377. [PubMed: 10440746]

51. Bonnesen B, Pappot H, Holmstav J, Skov BG. Vascular endothelial growth factor A and vascular endothelial growth factor receptor 2 expression in non-small cell lung cancer patients: relation to prognosis. Lung Cancer. 2009; 66:314–318. [PubMed: 19324448]
52. Chen F, Takenaka K, Ogawa E, et al. Flt-4-positive endothelial cell density and its clinical significance in non-small cell lung cancer. Clin Cancer Res. 2004; 10:8548–8553. [PubMed: 15623638]
53. Takenaka K, Katakura H, Chen F, et al. The ratio of membrane-bound form *Flt-1* mRNA to *VEGF* mRNA correlates with tumor angiogenesis and prognosis in non-small cell lung cancer. Cancer Lett. 2007; 246:34–40. [PubMed: 16530326]
54. Kim, ES.; Herbst, RS.; Lee, JJ.; Blumenschein, GR., Jr; Tsao, A.; Alden, CM.; Tang, X.; Liu, S.; Stewart, DJ.; Heymach, JV.; Tran, HT.; Hicks, ME.; Erasmus, J., Jr; Gupta, S.; Powis, G.; Lippman, SM.; Wistuba, II.; Hong, WK. The BATTLE trial (Biomarker-integrated Approaches of Targeted Therapy for Lung Cancer Elimination): personalizing therapy for lung cancer. Presented at: the 101st Annual Meeting of the American Association for Cancer Research; April 17–21, 2010; Washington, DC. 2010.
55. Azim HA Jr, Ganti AK. Targeted therapy in advanced non-small cell lung cancer (NSCLC): where do we stand? Cancer Treat Rev. 2006; 32:630–636. [PubMed: 17034953]
56. Herbst RS, Blumenschein GR Jr, Kim ES, et al. Sorafenib treatment efficacy and KRAS biomarker status in the Biomarker-Integrated Approaches of Targeted Therapy for Lung Cancer Elimination (BATTLE) trial. J Clin Oncol (Meeting Abstracts). 2010; 28:7609.
57. Alvarez RH, Kantarjian HM, Cortes JE. Biology of platelet-derived growth factor and its involvement in disease. Mayo Clin Proc. 2006; 81:1241–1257. [PubMed: 16970222]
58. Bergsten E, Uutela M, Li X, et al. PDGF-D is a specific, protease-activated ligand for the PDGF β-receptor. Nat Cell Biol. 2001; 3:512–516. [PubMed: 11331881]
59. Andrae J, Gallini R, Betsholtz C. Role of platelet-derived growth factors in physiology and medicine. Genes Dev. 2008; 22:1276–1312. [PubMed: 18483217]
60. Abramsson A, Lindblom P, Betsholtz C. Endothelial and nonendothelial sources of PDGF-B regulate pericyte recruitment and influence vascular pattern formation in tumors. J Clin Invest. 2003; 112:1142–1151. [PubMed: 14561699]
61. Jain RK, Booth MF. What brings pericytes to tumor vessels? J Clin Invest. 2003; 112:1134–1136. [PubMed: 14561696]
62. Crawford Y, Kasman I, Yu L, et al. PDGF-C mediates the angiogenic and tumorigenic properties of fibroblasts associated with tumors refractory to anti-VEGF treatment. Cancer Cell. 2009; 15:21–34. [PubMed: 19111878]
63. Kubo T, Piperdi S, Rosenblum J, et al. Platelet-derived growth factor receptor as a prognostic marker and a therapeutic target for imatinib mesylate therapy in osteosarcoma. Cancer. 2008; 112:2119–2129. [PubMed: 18338812]
64. Pasanisi P, Venturelli E, Morelli D, Fontana L, Secreto G, Berrino F. Serum insulin-like growth factor-I and platelet-derived growth factor as biomarkers of breast cancer prognosis. Cancer Epidemiol Biomarkers Prev. 2008; 17:1719–1722. [PubMed: 18628423]
65. Nakamura Y, Tanaka F, Yoshikawa Y, et al. PDGF-BB is a novel prognostic factor in colorectal cancer. Ann Surg Oncol. 2008; 15:2129–2136. [PubMed: 18478301]
66. Korc M, Friesel RE. The role of fibroblast growth factors in tumor growth. Curr Cancer Drug Targets. 2009; 9:639–651. [PubMed: 19508171]
67. Seghezzi G, Patel S, Ren CJ, et al. Fibroblast growth factor-2 (FGF-2) induces vascular endothelial growth factor (VEGF) expression in the endothelial cells of forming capillaries: an autocrine mechanism contributing to angiogenesis. J Cell Biol. 1998; 141:1659–1673. [PubMed: 9647657]
68. Beenken A, Mohammadi M. The FGF family: biology, pathophysiology and therapy. Nat Rev Drug Discov. 2009; 8:235–253. [PubMed: 19247306]
69. Casanovas O, Hicklin DJ, Bergers G, Hanahan D. Drug resistance by evasion of antiangiogenic targeting of VEGF signaling in late-stage pancreatic islet tumors. Cancer Cell. 2005; 8:299–309. [PubMed: 16226705]

70. Presta M, Dell'Era P, Mitola S, Moroni E, Ronca R, Rusnati M. Fibroblast growth factor/fibroblast growth factor receptor system in angiogenesis. Cytokine Growth Factor Rev. 2005; 16:159–178. [PubMed: 15863032]
71. Donnem T, Al-Shibli K, Al-Saad S, Busund LT, Bremnes RM. Prognostic impact of fibroblast growth factor 2 in non-small cell lung cancer: coexpression with VEGFR-3 and PDGF-B predicts poor survival. J Thorac Oncol. 2009; 4:578–585. [PubMed: 19318994]
72. Iwasaki A, Kuwahara M, Yoshinaga Y, Shirakusa T. Basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) levels, as prognostic indicators in NSCLC. Eur J Cardiothorac Surg. 2004; 25:443–448. [PubMed: 15019676]
73. Hsu I-L, Su W-C, Yan J-J, Chang J-M, Lai W-W. Angiogenetic biomarkers in non-small cell lung cancer with malignant pleural effusion: correlations with patient survival and pleural effusion control. Lung Cancer. 2009; 65:371–376. [PubMed: 19157636]
74. Fish JE, Srivastava D. MicroRNAs: opening a new vein in angiogenesis research. Sci Signal. 2009; 2:e1.
75. Ortholan C, Puissegur MP, Ilie M, Barbry P, Mari B, Hofman P. MicroRNAs and lung cancer: new oncogenes and tumor suppressors, new prognostic factors and potential therapeutic targets. Curr Med Chem. 2009; 16:1047–1061. [PubMed: 19275611]
76. Patnaik SK, Kannisto E, Knudsen S, Yendamuri S. Evaluation of microRNA expression profiles that may predict recurrence of localized stage I non-small cell lung cancer after surgical resection. Cancer Res. 2010; 70:36–45. [PubMed: 20028859]
77. Yanaihara N, Caplen N, Bowman E, et al. Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. Cancer Cell. 2006; 9:189–198. [PubMed: 16530703]
78. Yu SL, Chen HY, Chang GC, et al. MicroRNA signature predicts survival and relapse in lung cancer. Cancer Cell. 2008; 13:48–57. [PubMed: 18167339]
79. Raponi M, Dossey L, Jatkoe T, et al. MicroRNA classifiers for predicting prognosis of squamous cell lung cancer. Cancer Res. 2009; 69:5776–5783. [PubMed: 19584273]
80. Murugaiyan G, Saha B. Protumor vs antitumor functions of IL-17. J Immunol. 2009; 183:4169–4175. [PubMed: 19767566]
81. Waugh DJ, Wilson C. The interleukin-8 pathway in cancer. Clin Cancer Res. 2008; 14:6735–6741. [PubMed: 18980965]
82. Yuan A, Chen JJW, Yao P-L, Yang P-C. The role of interleukin-8 in cancer cells and microenvironment interaction. Front Biosci. 2005; 10:853–865. [PubMed: 15569594]
83. Orditura M, De Vita F, Catalano G, et al. Elevated serum levels of interleukin-8 in advanced non-small cell lung cancer patients: relationship with prognosis. J Interferon Cytokine Res. 2002; 22:1129–1135. [PubMed: 12513912]
84. Yuan A, Yang P-C, Yu C-J, et al. Interleukin-8 messenger ribonucleic acid expression correlates with tumor progression, tumor angiogenesis, patient survival, and timing of relapse in non-small-cell lung cancer. Am J Respir Crit Care Med. 2000; 162:1957–1963. [PubMed: 11069840]
85. Chen X, Wan J, Liu J, et al. Increased IL-17-producing cells correlate with poor survival and lymphangiogenesis in NSCLC patients. Lung Cancer. 2009
86. Voest EE, Kenyon BM, O'Reilly MS, Truitt G, D'Amato RJ, Folkman J. Inhibition of angiogenesis in vivo by interleukin 12. J Natl Cancer Inst. 1995; 87:581–586. [PubMed: 7538593]
87. Nikolinakos PG, Altorki N, Yankelevitz D, et al. Plasma cytokine and angiogenic factor profiling identifies markers associated with tumor shrinkage in early-stage non-small cell lung cancer patients treated with pazopanib. Cancer Res. 2010; 70:2171–2179. [PubMed: 20215520]
88. Krysan K, Reckamp KL, Sharma S, Dubinett SM. The potential and rationale for COX-2 inhibitors in lung cancer. Anticancer Agents Med Chem. 2006; 6:209–220. [PubMed: 16712449]
89. Wheeler-Jones CPD, Farrar C, Garonna E. Protease-activated receptors, cyclo-oxygenases and pro-angiogenic signalling in endothelial cells. Biochem Soc Trans. 2009; 37:1179–1183. [PubMed: 19909242]
90. Tsubochi H, Sato N, Hiyama M, et al. Combined analysis of cyclooxygenase-2 expression with p53 and Ki-67 in nonsmall cell lung cancer. Ann Thorac Surg. 2006; 82:1198–1204. [PubMed: 16996907]

91. Yuan A, Yu C-J, Shun C-T, et al. Total cyclooxygenase-2 mRNA levels correlate with vascular endothelial growth factor mRNA levels, tumor angiogenesis and prognosis in non-small cell lung cancer patients. Int J Cancer. 2005; 115:545–555. [PubMed: 15704107]
92. Timotheadou E, Skarlos DV, Samantas E, et al. Evaluation of the prognostic role of a panel of biomarkers in stage IB-IIIA non-small cell lung cancer patients. Anticancer Res. 2007; 27:4481–4489. [PubMed: 18214064]
93. Nakashima T, Huang C, Liu D, et al. Neural-cadherin expression associated with angiogenesis in non-small-cell lung cancer patients. Br J Cancer. 2003; 88:1727–1733. [PubMed: 12771988]
94. Gogali A, Charalabopoulos K, Zampira I, et al. Soluble adhesion molecules E-cadherin, ICAM-1 and E-selectin as lung cancer biomarkers. Chest. 2010
95. Guney N, Soydinc HO, Derin D, et al. Serum levels of intercellular adhesion molecule ICAM-1 and E-selectin in advanced stage non-small cell lung cancer. Med Oncol. 2008; 25:194–200. [PubMed: 18008189]
96. Chang H, Iizasa T, Shibuya K, et al. Increased expression of collagen XVIII and its prognostic value in nonsmall cell lung carcinoma. Cancer. 2004; 100:1665–1672. [PubMed: 15073855]
97. Iizasa T, Chang H, Suzuki M, et al. Overexpression of collagen XVIII is associated with poor outcome and elevated levels of circulating serum endostatin in non-small cell lung cancer. Clin Cancer Res. 2004; 10:5361–5366. [PubMed: 15328173]
98. Yoo J, Jung JH, Lee MA, et al. Immunohistochemical analysis of non-small cell lung cancer: correlation with clinical parameters and prognosis. J Korean Med Sci. 2007; 22:318–325. [PubMed: 17449943]
99. West H, Lilenbaum R, Harpole D, Wozniak A, Sequist L. Molecular analysis-based treatment strategies for the management of non-small cell lung cancer. J Thorac Oncol. 2009; 4:S1029–S1039. [PubMed: 19704347]
100. Bernaards C, Hegde P, Chen D, et al. Circulating vascular endothelial growth factor (VEGF) as a biomarker for bevacizumab-based therapy in metastatic colorectal, non-small cell lung, and renal cell cancers: Analysis of phase III studies. J Clin Oncol. 2010; 28 Abstract 10519.

Table 1

Studies of the Prognostic Value of VEGF in NSCLC

Study population	Expression type	Key findings
1,816 patients treated within phase III trials of bevacizumab for NSCLC, colorectal cancer, or renal cell carcinoma[100]	Plasma VEGF—pretreatment	Association between higher circulating VEGF (albeit not well reflective of tumor VEGF expression) and shortened progression-free and overall survival regardless of bevacizumab treatment
55 patients treated with postoperative radiotherapy but not chemotherapy for stage I–IIIA NSCLC[48]	VEGF—tumor and stroma	Univariate: significant correlation between high tumor cell VEGF-A expression and poor survival (P=0.004); stromal VEGF-C expression was borderline significant (P=0.066) Multivariate: VEGF expression not significant prognosticator
130 patients who underwent complete resection for stage I–III NSCLC[29]	VEGF—tumor	Multivariate: VEGF-A expression not significant prognosticator
153 patients surgically treated for stage I–IIIB NSCLC[24]	VEGF—tumor	Correlation between tumor hypervascularity and expression of VEGF-A (P=0.0442) but not VEGF-C Multivariate: VEGF-A was a significant prognosticator (RR, 2.012; P=0.0101), especially in the adenocarcinoma subset (RR, 3.816; P=0.0025), whereas VEGF-C was a significant prognosticator in the squamous cell subset (RR, 3.946; P=0.0143)
451 patients with previously untreated NSCLC who subsequently received standard treatment (without anti-VEGF therapy)[28]	Plasma VEGF—pretreatment	Circulating VEGF levels varied significantly with disease stage (P=0.01), and patients with lymph node involvement (P=0.01) or poor performance status (P <0.0001) were more likely to have high serum levels of VEGF Univariate: significant correlation between high pretreatment serum VEGF level and poor survival (P=0.0002) Multivariate: pretreatment serum VEGF level not significant prognosticator

NSCLC, non–small-cell lung cancer; RR, relative risk; VEGF, vascular endothelial growth factor.

Table 2

Studies of the Prognostic Value of VEGFR in NSCLC

Study population	Expression type	Key findings
102 chemotherapy-naive patients surgically treated for stage I–IIIB NSCLC[51]	VEGFR-2—tumor and stroma	No significant difference in survival between patients with VEGFR-2-positive versus VEGFR-2-negative tumors
55 patients treated with postoperative radiotherapy but not chemotherapy for stage I–IIIA NSCLC[48]	VEGFR-1,-2,-3—tumor and stroma	Univariate: significant correlation between high tumor cell VEGFR-1 (P=0.028), VEGFR-2 (P=0.021), and VEGFR-3 (P=0.001) expression and poor survival Multivariate: VEGFR expression not significant prognosticator
335 patients surgically treated for stage I–IIIA NSCLC[47]	VEGFR-1,-2,-3—tumor and stroma	Univariate: significant correlation between tumor cell VEGFR-1 (P=0.013), VEGFR-2 (P=0.006), and VEGFR-3 (P=0.0003) expression as well as high stromal cell VEGFR-1 (P=0.01) and VEGFR-2 (P=0.019) and disease-specific survival Multivariate: tumor cell VEGFR-3 expression was a significant prognosticator (P=0.007); similar trend observed for tumor VEGFR-2 expression (P=0.085)
60 patients surgically treated for stage I NSCLC (without induction or adjuvant chemotherapy)[49]	VEGFR-1,-2—tumor	Univariate: significant correlation between VEGFR-2 expression (P=0.05) but not VEGFR-1 expression and survival Multivariate: Tumor positivity for both VEGFR-1 and VEGFR-2 was a significant prognosticator (HR, 4.79; P=0.002)
180 patients who underwent complete resection for stage I–IIIB NSCLC (without induction chemotherapy or radiation)[46]	VEGFR-3—tumor	Association between significantly lower survival rates for patients with VEGFR-3-positive versus VEGFR-3-negative tumors (P<0.001) Univariate: significant correlation between VEGFR-3 expression and survival (P<0.01) Multivariate: VEGFR-3 expression was the sole independent prognosticator (P<0.01)
69 patients surgically treated for stage I–II NSCLC[50]	VEGFR-1,-2—tumor, stroma, and endothelial cells	No correlation between VEGFR-1 or VEGFR-2 expression and survival

HR, hazard ratio; NSCLC, non–small-cell lung cancer; VEGFR, vascular endothelial growth factor receptor.