Stromal Expression of VEGF-A and VEGFR-2 in Prostate Tissue Is Associated With Biochemical and Clinical Recurrence After Radical Prostatectomy

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BACKGROUND. There is probably significant overtreatment of patients with prostate cancer due to a lack of sufficient diagnostic tools to predict aggressive disease. Vascular endothelial growth factors (VEGFs) and their receptors (VEGFRs) are potent mediators of angiogenesis and tumor proliferation, but have been examined to a limited extent in large prostate cancer studies. Meanwhile, recent promising results on VEGFR-2 inhibition have highlighted their importance, leading to the need for further investigations regarding their expression and prognostic impact.

DESIGN. Using tissue microarray and immunohistochemistry, the expression of VEGFs (VEGF-A and VEGF-C) and their receptors (VEGFR-2 and VEGFR-3) were measured in neoplastic tissue and corresponding stroma from radical prostatectomy specimens in 535 Norwegian patients. Their expression was evaluated semiquantatively and associations with event-free survival were calculated.

RESULTS. High expression of VEGFR-2 in either stroma or epithelium was independently associated with a higher incidence of prostate cancer relapse (HR = 4.56, $P = 0.038$). A high combined expression of either VEGF-A, VEGFR-2 or both in stroma was independently associated with a higher incidence of biochemical failure (HR = 1.77, $P = 0.011$).

CONCLUSIONS. This large study highlights the prognostic importance of VEGF-A and VEGFR-2 stromal expression. Analyses of these biomarkers may help distinguish which patients will benefit from radical treatment. Together with previous studies showing efficiency of targeting VEGFR-2 in prostate cancer, this study highlights its potential as a target for therapy, and may aid in future selection of prostate cancer patients for novel anti-angiogenic treatment. *Prostate 75:1682–1693, 2015.* © 2015 Wiley Periodicals, Inc.

KEY WORDS: **prostate cancer; veg; angiogenesis; tissue microarray; immunohistochemistry**

INTRODUCTION

Prostate cancer (PC) is the most frequent cancer in men, and the second most common cause of male cancer death in developed countries [1]. However, once diagnosed with PC, the mortality of PC is estimated to be only 2–3%. The challenge is to

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distinguish between patients with an aggressive and potentially deadly form of PC, versus patients with more indolent disease.

Clinical prognostic risk stratification using preoperative PSA value, cTNM and Gleason score are well-established, but imprecise. This results in a significant overtreatment (radical therapy), but possibly also undertreatment of some patients [2–4]. There is a need for better prognostic tools to aid in the prediction of which patients will benefit from curative treatment.

Angiogenesis is a well-studied hallmark of cancer [5]. Without sufficient blood flow, the malignant tumor cannot grow to a self-sustaining tumor of significant size. The vascular endothelial growth factor-A (VEGF-A) is a central regulator of tumor--induced angiogenesis and is critical for tumor growth and metastasis [6]. The vascular endothelial growth factor receptor-2 (VEGFR-2) plays an important role in angiogenesis, endothelial cell proliferation, migration, and survival.

VEGF-A overexpression has been associated with tumor progression and poor prognosis in colorectal carcinoma [7], breast cancer [8], lung cancer, [9] and in squamous cell carcinoma of the head and neck [10]. For prostate cancer, the few previous clinicopathological studies of VEGF expression have not yielded consistent results. Few previous studies have evaluated the expression of VEGF-A in epithelium and its association to relapse from PC [11–15]. Stromal expression of VEGF-A in PC has hardly been studied. Wu et al. observed that high Gleason grade tumors and advanced disease had significantly higher frequency of VEGF expression in stroma but not in glandular epithelium [16]. However, two recent studies found no association between VEGF-A expression and PC relapse [17,18]. VEGFR-2 is known to be expressed in vascular endothelium, particularly enriched for neoangiogenesis with cancer [19].

In a randomized phase 2 study, the MET/VEGFR-2 inhibitor cabozantinib led to reduced pain in 57% of patients with metastatic castration-resistant prostate cancer (mCRPC) [20], but preliminary results failed to show improvement in overall survival in the phase 3 study COMET-1 [21]. In addition, the anti-angiogenic drug tasquinimod has also showed encouraging results in a phase 2 study [22]. Also, tasquinimod reduced the risk of radiographic cancer progression and death compared to placebo in men with mCRPC. However, the drug did not extend overall survival [23]. The VEGFR-2 inhibitor ramucirumab inhibited cell proliferation in vitro, as well as tumor progression in mouse xenograft models of human cancer. A phase 2 study in prostate cancer found ramucirumab to have encouraging results, but to our knowledge the results have so far only been published as an abstract [24]. Ramucirumab was recently approved by the FDA as treatment for advanced non-small cell lung cancer.

As previous studies have shown conflicting results, we systematically investigated both tumor and stromal expression of the anti-angiogenic ligands VEGF-A and VEGF-C, and their respective receptors VEGFR-2 and VEGFR-3 as biomarkers in a large cohort of 535 prostatectomized patients. Herein, we explored the associations with clinical outcome in terms of biochemical recurrence, clinical recurrence, and death from PC.

MATERIALS AND METHODS

Patients

671 patients who underwent radical prostatectomy with curative intent for adenocarcinoma in the prostate from 1995 to 2005 were retrospectively identified from the Departments of Pathology at the University Hospital of Northern Norway (n = 267), Nordland Hospital (n = 63), St. Olavs Hospital (n = 330) and Levanger Hospital (n = 11). Of these, 136 patients were excluded due to (i) previous non-superficial cancer within 5 years of PC diagnosis (n = 4), (ii) radiotherapy to the pelvis prior to surgery (n = 1), (iii) inadequate paraffin-embedded tissue blocks (n = 130), and (iv) lack of follow-up data (n = 1), leaving a total of 535 patients included in the study. None of the patients had received pre-operative hormonal therapy. The cohort is thoroughly described in a previous paper [25].

We collected relevant data from medical journals: Demographical data, age at surgery, previous medical history, retropubic, or perineal surgery, and preoperative PSA measured immediately before surgery. Further, we collected data until the last follow-up date (31.12.12) or until patients' death. The patients' clinical outcome was recorded for a median follow-up of 7.4 years (range 0.5–16 years). These data were: Postoperative PSA values, as well as postoperative therapy (radio-, hormonal, and/or chemotherapy). The following endpoints were used: Biochemical failure (BF) defined as postoperative PSA $\geq$0.4 or intervention with adjuvant therapy; Clinical failure (CF) defined as clinically palpable tumor recurrence in the prostate bed or metastasis verified by radiology; Prostate cancer specific death (PCD), defined as death caused by PC stated in the patients' journal.

Tissues and Tissue Microarray Construction

Tumor tissues, consisting of formalin-fixed paraffin-embedded blocks of prostate tissue from the

patients' prostatectomies, were collected from the archives of the pathological departments. One experienced pathologist (E.R.) reevaluated the prostate samples and classified them according to the updated WHO guidelines [26,27]. Two pathologists (E.R. and L.T.B.) identified the most representative areas of cancer epithelium cells and tumor-near stroma. Each area was biopsied with at least two 0.6 mm cores. In addition, two biopsies from normal tissue of each patient were also sampled. The cores were arranged in tissue microarray (TMA) blocks for large-scale analysis. To include all core samples, TMA blocks were constructed. Multiple 4 μm sections were cut with a Micron microtone (HM355S), affixed to glass slides and stained by specific antibodies for immunohistochemical analysis (IHC). The detailed methodology has been reported previously [28].

Immunohistochemistry

The antibodies used were VEGF-A rabbit polyclonal (Thermo–Fisher; cat.no AB-9031; 1:50 dilution), VEGF-C rabbit polyclonal (Invitrogen; cat.no 18-2255; 1:25 dilution), VEGFR-2 rabbit monoclonal (Cell Signaling Technology; clone 55B11; cat.no #2479; 1:100 dilution) and VEGFR-3 mouse monoclonal (Merck Millipore; clone 9D9F9; cat.no MAB-3757; 1:100 dilution). VEGF-A, VEGF-C and VEGFR-2 were stained manually with the Dako EnVision detection kit (Dako, Glostrup, Denmark). In brief, after drying overnight, the slides were deparaffinized in xylene and dehydrated with alcohols. Endogenous peroxidase activity was inhibited by incubating the sections in 1.5% H_2O_2 for 10 min, and antigen retrieval for primary antibodies was done by placing the specimens in 0.01 mol/L citrate buffer (pH 6.0) and exposing them to two repeated microwave heatings of 10 min at 450 W. Nonspecific binding sites were blocked by 10% normal goat serum for 30 min. The sections were incubated with primary antibodies overnight, and then incubated with the secondary antibody (Dako Real Envision/HRP, K5007) for 30 min. Sections were counterstained with hematoxylin and mounted for examination with light microscope.

VEGFR-3 was stained using the automated BenchMark XT stainer (Ventana Medical Systems, Inc., Tucson, AZ). Epitope retrieval was accomplished on the automated stainer with CC1 solution (Ventana Medical Systems, Inc., Tucson, AZ). The VEGFR-3 antibody was incubated for 32 min and was detected by using the iVIEW DAB Detection Kit (Ventana Medical Systems, Inc., Tucson, AZ). Finally, to visualize the nuclei, the slides were counterstained with Ventana Hematoxylin II reagent for 8 min, followed by a Bluing reagent for 4 min.

For validation, two different controls for our staining method were applied. First, control staining of the sections with an isotype-matched control antibody without the primary antibody. Secondly, multiple organ tissue microarray as positive and negative tissue controls were used to verify the specificity. The positive tissue controls comprised of human angiosarcoma for VEGF-A and VEGFR-2, colon carcinoma for VEGF-C and lymph node for VEGFR-3.

Scoring of Immunohistochemistry

The IHC stained TMA slides were scanned and digitalized using the ARIOL imaging system (Applied Imaging Corp., San Jose, CA), and uploaded into the ARIOL software. Two pathologists (E.R, S.A-S.) independently and semiquantatively scored viable parts of each anonymized core by light microscopy. The pathologists were blinded for each other's score. Each core was scored by the dominant intensity of staining: 0 = no staining; 1 = weak staining; 2 = moderate staining; 3 = strong staining. The core was scored as "missing" if the core was missing or considered of insufficient quality to score by both observers. A final score for marker expression in both tumor epithelium (tumor) and tumor-near stroma (stroma) for each patient was calculated using the mean values of the observers' scoring of the patients cores. Scoring of IHC cores were dichotomized to low and high expressions. Cut-off values were chosen in order to secure statistically sufficient numbers in each group. In general, there was a low expression of VEGF-A in the tumor stromal areas (cut-off 0.63). For VEGFR-2, there was a high expression in tumor stromal areas (cut-off 2.17), and a low expression in tumor epithelial areas (cut-off 0.7).

Statistical Methods

SPSS 21.0.0 (Chicago, IL) was used for all statistical analyses. Correlations were analyzed using Spearman's rank correlation coefficient. Univariate survival analyses were done by the Kaplan–Meier method, and the statistical significant difference between survival curves was assessed by the log-rank test. Presentation of the survival curves were terminated at 134 months, due to less than 10% of patients at risk after this point. The significance level (P-value) was not corrected for multiply hypotheses testing, due to a relatively large number of patients and few hypotheses giving little chance for Type I errors. For multivariate analyses, the backward conditional Cox-regression analysis was used with a probability for stepwise entry at 0.05 and stepwise removal of 0.10. A $P < 0.05$ was considered statistically significant for all analyses.

Ethics

The study has been approved by The Regional Committee for Medical and Health Research Ethics (2009/1393), the Data Protection Official for Research (NSD), and the National Data Inspection Board.

RESULTS

Clinicopathological Variables and Patient Characteristics

The patients' clinicopathological data are presented in the first part of Table I. Median age at surgery was 62 (47–75). The prostatectomies were retropubic in 81% of cases, and perineal in 19% of cases. At the last follow-up, 32% of the patients had BF, 6.7% of the patients had CF, and 2.8% of the patients had PCD. Median PSA was 8.8 (range 0.7–104) and the median tumor size was 20 mm (2.0–50).

Expressions and Correlations

The staining of VEGF-A was both nuclear and cytoplasmic. There was generally a low expression of VEGF-A in tumor stromal areas compared with VEGFR-2, which was strongly expressed. The staining intensity of VEGF-C was restricted to granular cytoplasmic staining in a few endothelial cells. For VEGFR-3 there was a strong nuclear staining intensity and a weaker cytoplasmic expression. Representative light microscopic examples of normal tissue as well as weak and strong expression of VEGF-A and VEGFR-2 in epithelium and stroma are shown in Figure 1. None of the biomarkers or their combinations had any direct correlation to any of the clinicopathological variables.

In the control cores, there was in general no expression of VEGF-A and VEGFR-2 in normal epithelium or stroma (Fig. 1). VEGFR-2 was expressed in vascular endothelium in both normal and cancerous prostate specimen, as expected from previous studies [29].

In 45% of the cases where VEGF-A was highly expressed in stroma, epithelium was also highly expressed, leaving 55% of the cases where high expression occurred in the stroma alone. Besides, there was no significant correlation between positive VEGF-A staining in the epithelium versus stroma ($P = 0.074$).

For VEGFR-2, high epithelial expression was observed along with high stromal expression in 55% of the cases, leaving 45% of the cases with high expression in stroma alone. There was no significant correlation between positive VEGFR-2 staining in epithelium versus stroma ($P = 0.184$).

Based on the staining distribution and the absence of correlation between epithelial and stromal staining, the IHC staining was considered to be specific. Besides, there was no expression of VEGF-A and VEGFR-2 in control cores of normal prostate tissue.

Univariate Analyses

Results for the clinicopathological variables are presented in Table I. For BF, significant prognostic factors were pT-stage ($P < 0.001$), pN-stage ($P < 0.001$), preoperative PSA ($P < 0.001$), Gleason score ($P < 0.001$), tumor size ($P < 0.001$), perineural infiltration ($P < 0.001$), positive surgical margin [($P = 0.040$); subclasses: apical ($P = 0.042$) and non-apical margins ($P < 0.001$)] and vascular infiltration ($P < 0.001$). For CF, significant prognostic factors were pT-stage ($P < 0.001$), pN-stage ($P < 0.001$), Gleason score ($P < 0.001$), tumor size ($P < 0.013$), perineural infiltration ($P < 0.001$), positive surgical margin [($P = 0.031$); with subclass non-apical margin ($P < 0.001$)] and vascular infiltration ($P < 0.001$). The significant prognostic factors for PCD were pT-stage ($P = 0.027$), pN-stage ($P < 0.001$), Gleason score ($P < 0.001$), perineural infiltration ($P = 0.002$), non-apical positive surgical margin ($P = 0.029$) and vascular infiltration ($P = 0.009$).

Results from the univariate analyses of molecular markers according to BF, CF and PCD endpoints are presented in Table I and Figures 2, 3 and 4. Patients with high expression of VEGF-A in stroma ($P = 0.013$), high expression of VEGFR-2 in stroma ($P = 0.032$) and a combination of high expression of either VEGF-A or VEFGR-2 in stroma ($P = 0.003$) had significantly worse outcome regarding BF. For CF, patients with high expression of VEGFR-2 in stroma ($P = 0.031$) and high expression of VEGFR-2 in either stroma, epithelium or both ($P = 0.029$) had a significantly worse outcome. None of the markers were significantly associated with worse outcome regarding PCD, though VEGFR-2 tended towards significance ($P = 0.076$).

Univariate analyses of VEGF-C and VEGFR-3 expressions showed no significant differences in BF, CF and PCD.

Multivariate Analyses

Results from two of three multivariate models regarding clinicopathological variables and biomarkers are shown in Table II. Three models were calculated as it is prohibited to analyze combinations of the same marker in one Cox regression model. Model 1 shows that besides clinicopathological variables [pT-status ($P < 0.001$), Gleason ($P = 0.010$), positive non-apical margin ($P = 0.003$) and positive apical

TABLE I. Patient Characteristics, Clinicopathological Variables and Expressions of VEGF-A and VEGFR-2 in 535 Prostate Cancer Patients (univariate analyses; log-rank test)

			BF (n = 170)			CF (n = 36)			PCD (n = 15)		
	Patients		Mean	5 year		Mean	5 year		Mean	5 year	
Characteristics	(n)	(%)	EFS	EFS	*P*	EFS	EFS	*P*	EFS	EFS	*P*
Age					0.555			0.056			0.600
<65 years	357	67%	128	77%		179	97%		183	99%	
≥65 years	178	33%	122	70%		159	95%		169	100%	
pT-stage					<0.001			<0.001			0.027
pT2	374	70%	145	83%		183	98%		184	99%	
pT3a	114	21%	96	60%		165	94%		181	100%	
pT3b	47	9%	60	43%		144	86%		163	95%	
pN-stage					<0.001			<0.001			<0.001
NX	264	50%	131	79%		182	98%		185	100%	
N0	268	50%	118	71%		171	95%		180	99%	
N1	3	1%	23	0%		56	33%		97	100%	
Preoperative PSA					<0.001			0.063			0.061
<10	308	58%	138	80%		179	98%		184	99%	
>10	221	41%	110	67%		171	94%		178	99%	
Missing	6	1%	—			—					
Gleason					<0.001			<0.001			0.001
3 + 3	183	34%	127	83%		169	99%		173	100%	
3 + 4	220	41%	135	76%		172	96%		178	100%	
4 + 3	80	15%	108	69%		171	94%		175	99%	
4 + 4	19	4%	87	63%		156	95%		167	94%	
>8	33	6%	53	34%		134	87%		155	97%	
Tumor size					<0.001			0.013			0.098
≤20 mm	250	47%	138	82%		180	98%		183	99%	
>20 mm	285	53%	118	67%		170	94%		180	99%	
Perineural infiltration					<0.001			<0.001			0.002
No	401	75%	130	79%		175	98%		180	99%	
Yes	134	25%	101	60%		161	91%		175	99%	
Positive surgical margin					0.040			0.031			0.697
No	249	47%	136	81%		180	98%		183	99%	
Yes	286	53%	113	69%		171	95%		180	99%	
Non-apical positive surgical margin					<0.001			<0.001			0.029
No	381	71%	140	81%		182	98%		185	99%	
Yes	154	29%	92	57%		160	92%		176	99%	
Apical positive surgical margin					0.042			0.593			0.313
No	325	61%	124	73%		174	96%		180	99%	
Yes	210	39%	126	77%		176	96%		183	99%	
Vascular infiltration					<0.001			<0.001			0.009
No	492	92%	131	77%		178	97%		183	99%	
Yes	43	8%	79	46%		139	85%		160	97%	
Surgical procedure					0.232			0.383			0.581
Retropubic	435	81%	130	76%		175	96%		181	99%	
Perineal	100	19%	118	67%		173	98%		179	100%	
VEGF-A in stroma					0.013			0.890			0.357
Low	331	62%	134	76%		175	96%		180	99%	
High	148	28%	112	67%		169	96%		180	99%	
Missing	56	10%	—			—			—		
VEGFR-2 in stroma					0.032			0.031			0.076
Low	231	43%	132	77%		175	99%		179	100%	
High	248	46%	121	71%		173	94%		179	99%	
Missing	56	10%	—			—			—		

(*Continued*)

TABLE I. (*Continued*)

			BF (n = 170)			CF (n = 36)			PCD (n = 15)		
	Patients		Mean	5 year		Mean	5 year		Mean	5 year	
Characteristics	(n)	(%)	EFS	EFS	*P*	EFS	EFS	*P*	EFS	EFS	*P*
VEGF-A and VEGFR-2 in stroma					0.003			0.345			0.757
Both low	149	28%	138	81%		167	99%		171	100%	
Either VEGF-A or VEGFR-2 high	257	48%	123	70%		175	96%		182	99%	
Both high	68	13%	102	67%		167	93%		176	98%	
Missing	61	11%	—			—			—		
VEGFR-2 in stroma and epithelium					0.053			0.029			0.230
Both stroma and epithelium low	113	21%	125	83%		159	100%		161	100%	
Either stroma, epithelium or both high	344	64%	127	73%		174	95%		181	99%	
Missing	78	15%	—			—			—		

BF, biochemical failure; CF, clinical failure; PCD, prostate cancer death; EFS, event free survival in months

margin ($P = 0.003$)], a high VEGF-A expression in stroma correlates with increased BF (HR = 1.51, $P = 0.016$). In model 2 we computed a co-expression variable of VEGF-A and VEGFR-2. We found high expression of either VEGF-A or VEGFR-2 in stroma (HR = 1.77) or both (HR = 2.02) were significantly associated with increased BF ($P = 0.011$). Besides, the same clinicopathological variables that were significant in model 1 also came out significant in model 2. In addition, a third model was analyzed (not presented), in which the results revealed that a VEGFR-2 expression in either stroma, epithelium or both was associated with worse CF-free survival (HR = 4.56, $P = 0.038$).

DISCUSSION

The current results demonstrate that overexpression of VEGF-A and VEGFR-2 in prostate adenocarcinoma is independently and significantly associated with biochemical and clinical recurrence in PC patients treated by radical prostatectomy. In our cohort, the risk of biochemical failure is nearly doubled (HR 1.77) provided high expression of VEGF-A or VEGFR-2 in stroma, while the risk of clinical failure is quadrupled (HR 4.56) if VEGFR-2 is overexpressed in either tumor epithelium, stroma or both.

VEGFR-2 has so far been scarcely studied in clinicopathological studies, as the major focus has been on VEGF-A. Marker studies involving both tumor epithelium and tumor stroma are even more rare. Our data indicate that VEGFR-2 is a stronger prognosticator than VEGF-A, and particularly that overexpression in the tumor-near stroma is of great significance.

The strength of our study is the large number of patients, the long clinical follow-up and that both tumor epithelium and stroma have been examined, as opposed to previous studies. In contrast to RT-PCR techniques, IHC markers allow us to visualize and assess expressions of antibodies in both the epithelial and stromal compartments.

Despite the long clinical follow-up, a weakness of this study is the low numbers of clinical recurrence and prostate cancer specific deaths (36 and 15 events, respectively). This shows that larger studies and longer follow-up are needed to properly evaluate the significant endpoints.

Our data demonstrating that VEGF-A is a poor prognostic factor in prostate cancer is consistent with the majority of previous studies in this disease [11–15]. Interestingly, our results emphasize that it is the VEGF-A overexpression in the tumor--near stroma rather than the tumor epithelium that is of greatest importance. Corroborating our findings, Wu et al. investigated 51 radical prostatectomy specimens and observed that high Gleason grade tumors and advanced disease had a significantly higher frequency of VEGF-A expression in tumor--near stroma, than the tumor epithelium [16]. Importantly, Vergis and coworkers, studying prostate cancer tissues from 308 prostatectomized patients and 289 patients undergoing prostate biopsies prior to radiotherapy, reported that increased VEGF-A expression was significantly and independently associated with a reduced time to biochemical failure [14]. In a smaller cohort (n = 40), Peyromaure et al. found that VEGF-A expression was the most significant predictive factor of cancer progression after radical prostatectomy [15]. In a more recent

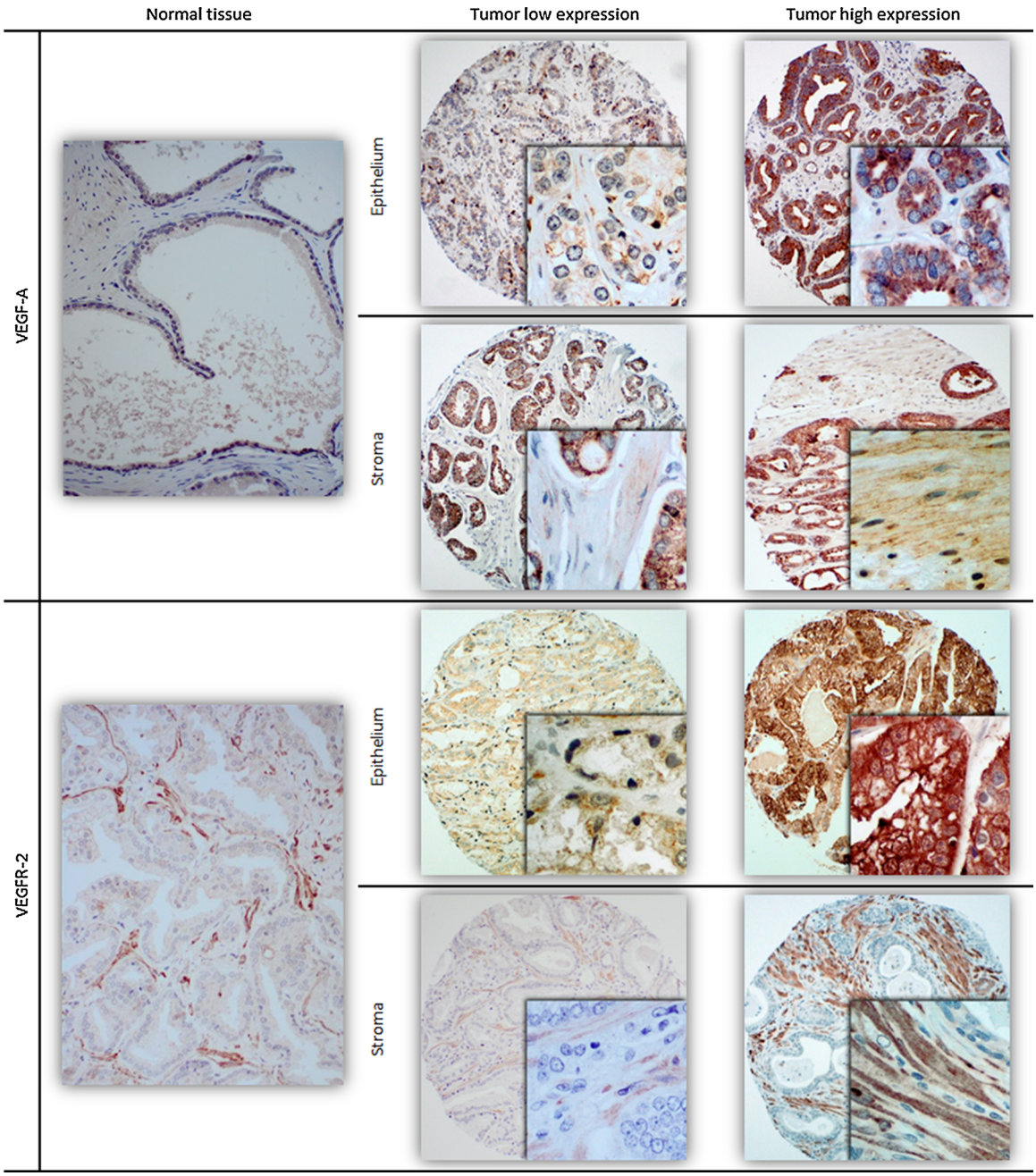

Fig. 1. Examples of low and high expressions of VEGF-A and VEGFR-2 immunohistochemical staining in tissue microarray cores of prostate cancer epithelium and stroma. 100x (main) and 400× (embedded) magnification.

investigation of 148 prostate cancer patient undergoing radical prostatectomy for clinically localized disease, Wang et al. found that high VEGF-A expression was more correlated to N+ prostate carcinoma and strongly predicted biochemical progression after prostatectomy [12]. In addition, Gravdal et al. reported that high vascular proliferation was significantly related to adverse clinicopathological features and was a strong and independent predictor for biochemical failure when investigating prostate cancer specimens from 104 cancer patients with localized disease [13]. However, stromal expression has not been specifically addressed in any of these studies.

Two recently published studies reported no association between VEGF-A expression and recurrence [17,18]. These studies were, however, of limited size, with shorter follow-up and without stromal assessments, emphasizing in particular the need for larger studies.

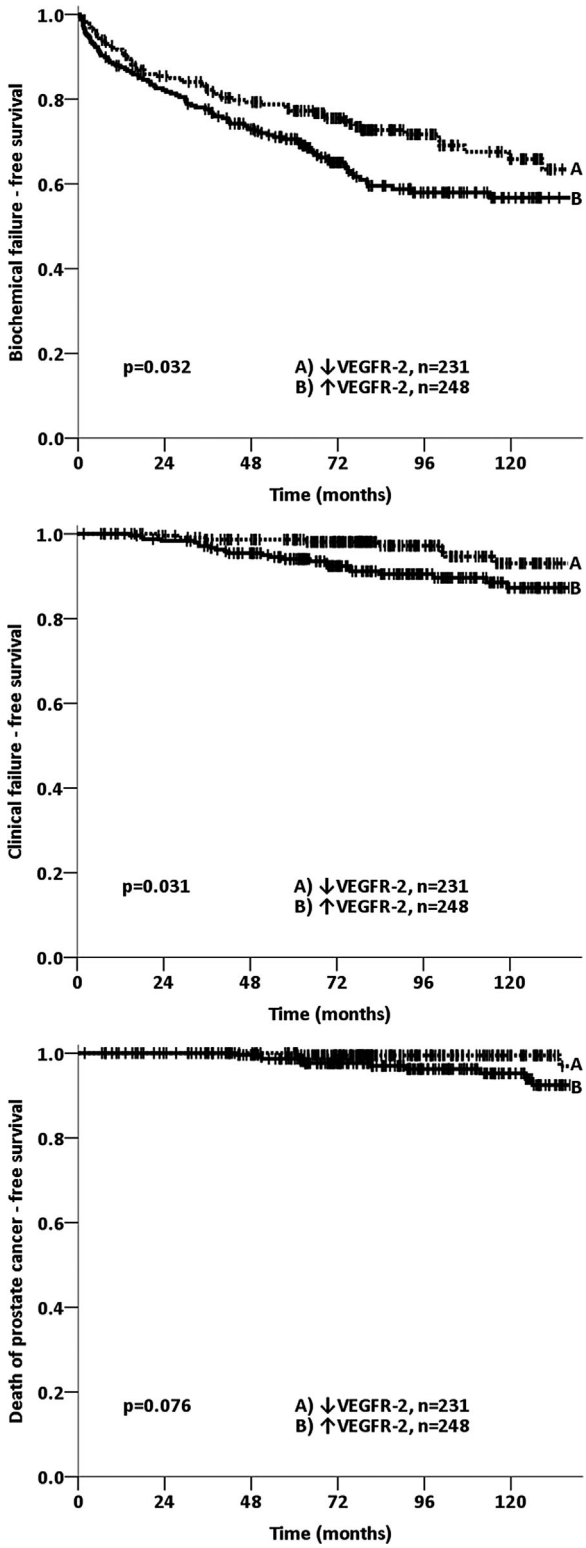

Fig. 2. Kaplan-Meier curves of low or high expression of VEGFR-2 in stroma for (top) biochemical failure, (middle) clinical failure and (bottom) death of prostate cancer.

The importance of our stromal findings appears biological plausible: The stromal microenvironment is an active and important biological component, as there is continuous and bilateral molecular crosstalk between normal cells and tumor cells of the stromal compartment, mediated through direct cell-cell contacts or by secreted molecules. Thus, minor changes in one compartment may cause dramatic alterations in the whole system [30].

The inhibition of angiogenic pathways is an established treatment for several common solid tumors. But its role in the management of prostate cancer is, however, still unclear. Several phase III studies of antiangiogenic agents in metastatic PC have yielded disappointing results: Adding the VEGF-A inhibitor bevacizumab to docetaxel chemotherapy in CRPC patients showed no significant improvement in overall survival, but led to increased toxicity and treatment related deaths [31]. Studies on sunitinib, the tyrosine kinase inhibitor (TKI) against VEGFR-2/platelet-derived growth factor receptor, in patients with advanced CRCP were discontinued due to ineffectiveness [32]. In a large randomized phase III study comparing docetaxel plus lenalidomide (an anti-angiogenic/immunomodulatory agent) versus docetaxel plus placebo, there was no improvement in overall survival in the experimental arm [33]. A recent phase II study of the VEGFR-targeting TKI pazopanib administered to 23 patients with CRPC failed to show sufficient activity in general to warrant further evaluation. Importantly, four patients had a long-term benefit, suggesting that targeting the VEGFR pathway may be highly relevant in selected patients, emphasizing the need for better predictive markers in these patients [34].

The rationale for further studies on antiangiogenic therapy remains strong as novel agents in this field have shown promising results. The dual VEGFR-2/MET targeting TKI cabozantinib has been shown to suppress angiogenesis, metastasis, and tumor growth in preclinical models, and led to significant survival benefits in a medullary thyroid cancer phase III study [35,36]. In a phase II non-randomized discontinuation trial for patients with mCRPC, cabozantinib yielded impressive palliation of bone pain and verified reduced bone metastases [20]. Although data showed encouraging symptomatic relief, preliminary results from the phase 3 trial COMET-1 did not show improvement in overall survival. Tasquinimod has been shown to decrease blood vessel density, though the exact mechanism of action is still unclear. In a randomized placebo-controlled phase II study in males with minimally symptomatic mCRPC, tasquinimod led to improved progression-free survival, and the treatment was well tolerated [22]. The phase III

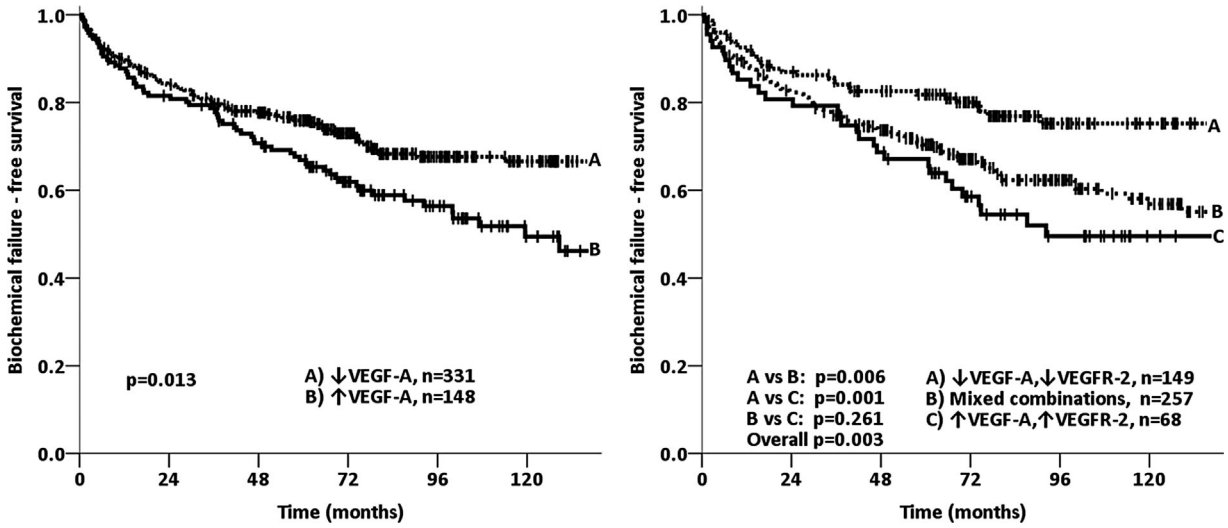

Fig. 3. Kaplan-Meier curves of (left) low or high expression of VEGF-A in stroma for biochemical failure, and (right) combinations of low and high expressions of VEGF-A and VEGFR-2 in stroma for biochemical failure.

trial failed, however, to improve in overall survival [23]. Preliminary results of a phase II study of the VEGFR-2 inhibitor ramucirumab plus mitoxantrone and prednisone in patients with mCRPC led to encouraging progression-free and overall survival [24]. PC is clinically and molecularly a heterogeneous disease and the lack of available predictive biomarkers for patient selection is apparently one of the key reasons why several large trials have produced disappointing results. Specific biomarkers associated with response to therapy are urgently needed to guide treatment selection among prostate cancer patients.

To our knowledge, targeting the VEGF-A/VEGFR-2 pathway is not previously studied in patients with localized PC. At the present, however, a randomized phase II trial of the VEGFR-1, -2 and -3 inhibitor axitinib, administered prior to surgery, is ongoing in high-risk prostate cancer [37]. Hence, the therapeutic combined inhibition of the VEGF-A/VEGFR-2 signaling may in the future be added to radical treatment of prostate cancer. Although first it will be necessary to further clarify the role of VEGF-A and VEGFR-2 in prostate cancer progression and relapse.

In conclusion, our results indicate that VEGF-A and VEGFR-2, primarily in stroma, are strong independent predictors of prostate cancer recurrence. With further validation of these results, VEGF-A and VEGFR-2 appear be important prognosticators and may in the future aid in treatment allocation of PC patients. As novel therapeutic agents such as

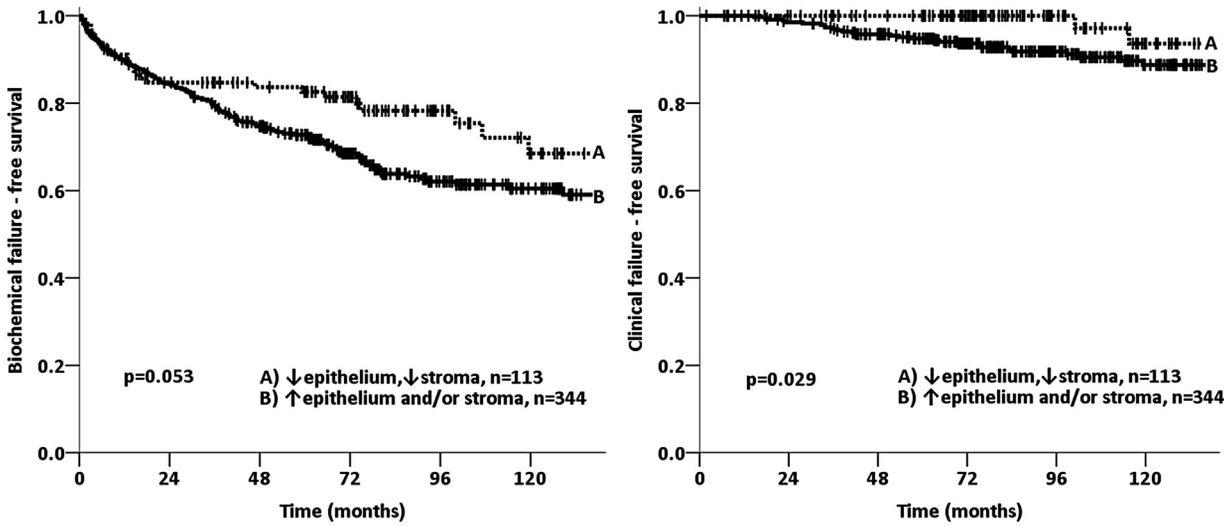

Fig. 4. Kaplan-Meier curves for low expressions of VEGFR-2 in stroma and epithelium versus high expression of VEGFR-2 in either stroma or epithelium or both for (left) biochemical failure and (right) clinical failure.

TABLE II. Expression of VEGF-A and VEGFR-2 in Prostate Tissue as Prognostic Factors in 535 Prostate Cancer Patients (multivariate analyses; Cox regression with backward conditional model)

Model 1	BF (n = 170)			CF (n = 36)		
Factor	HR	95 %CI	*P*	HR	95 %CI	*P*
pT status			<0.001			NE
pT2	1					
pT3a	1.87	1.27–2.76	0.002			
pT3b	2.59	1.58–4.24	<0.001			
Preoperative PSA > 10			NS			NE
Gleason			0.010			0.019
3+3	1			1		
3+4	1.09	0.72–1.65	0.684	2.68	0.84 - 8.61	0.097
4+3	1.65	1.03–2.64	0.036	3.80	1.10 - 13.1	0.034
4+4	1.95	0.92–4.13	0.081	3.52	0.64 - 19.5	0.149
> 8	2.55	1.41–4.61	0.002	7.79	2.33 - 26.0	0.001
Perineural infiltration			NS	2.29	1.09 - 4.85	0.030
Positive non-apical margin	1.70	1.20–2.42	0.003	0.40	0.19 - 0.84	0.016
Positive apical margin	0.59	0.41–0.83	0.003			NE
High expression of VEGF-A in stroma	1.51	1.08–2.10	0.016			NE
High expression of VEGFR-2 in stroma	1.32	0.95–1.84	0.094	1.98	0.90 - 4.36	0.088
Model 2*	BF (n = 170)			CF (n = 36)		
Factor	HR	95%CI	*P*	HR	95%CI	*P*
pT status			0.003			NE
pT2	1					
pT3a	1.69	1.13–2.53	0.011			
pT3b	2.26	1.35–3.77	0.002			
Preoperative PSA > 10	1.33	0.95–1.86	0.096			NE
Gleason			0.013			0.019
3+3	1			1		
3+4	1.07	0.71–1.62	0.751	2.45	0.87–6.90	0.090
4+3	1.63	1.08–2.62	0.042	2.87	0.91–9.10	0.073
4+4	1.92	0.91–4.05	0.086	2.73	0.52–14.2	0.223
> 8	2.57	1.39–4.73	0.003	6.74	2.21–20.6	0.001
Perineural infiltration			NS	2.48	1.23–5.04	0.012
Positive non-apical margin	1.74	1.22–2.48	0.002	3.22	1.56–6.64	0.002
Positive apical margin	0.58	0.41–0.83	0.003			NE
VEGF-A and VEGFR-2 in stroma			0.011			NE
Low expression of both	1					
High for either VEGF-A or VEGFR-2	1.77	1.14–2.58	0.009			
High expression of both	2.02	1.22–3.34	0.006			

BF, biochemical failure; CF, clinical failure; NE, not entered into Cox regression due to not significant in univariate analyses; NS, not significant and removed by backward model before last step of analyses.
*Two models are needed as it it prohibited to analyse combinations of the same marker in one analysis.

cabozantinib recently showed promising results in patients with CRPC, the VEGFR-2 axis appears to be of clinical importance from a therapeutic perspective.

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