Pathol. Oncol. Res. (2009) 15:193–201
DOI 10.1007/s12253-008-9107-z

ORIGINAL PAPER

Serum Levels of Angiogenic Factors and their Prognostic Relevance in Bladder Cancer

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Received: 6 May 2008 / Accepted: 2 September 2008 / Published online: 20 September 2008
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Abstract Angiogenesis plays a critical role in tumor growth. VEGF, angiopoietins (Ang-1, Ang-2) and their tyrosine kinase receptor Tie2 are major regulators of angiogenesis. The aim of this study was to evaluate the prognostic value of the serum levels of these factors in bladder cancer. We analyzed the serum samples of 117 bladder cancer patients and 64 healthy volunteers by enzyme linked immunosorbent assay (ELISA) for Ang-1, Ang-2, VEGF and the extracellular domain of Tie2. The statistical evaluation of the obtained data was performed via Kaplan–Meier log-rank test, univariate Cox analyses as well as Cox proportional hazards regression model. Serum Ang-1 levels of bladder cancer patients were significantly higher ($p<0.001$), while soluble Ang-2 and Tie2 levels were significantly lower ($p=0.016$ and $p=0.001$ respectively) in patients than those in controls. Cox univariate analysis revealed high sTie2 serum level as a risk factor for metastasis and as a borderline significant risk factor for disease related death ($p=0.022$ and $p=0.081$ respectively). These correlations were independent from tumor stage and grade in a Cox multivariate model ($p=0.016$ and $p=0.069$). These data indicate that the serum levels of analyzed angiogenic factors do change characteristically in bladder cancer. The soluble extracellular serum level of Tie2 may provide a stage and grade independent diagnostic tool to select a high risk group of bladder cancer patients.

Keywords Bladder cancer · Serum · Angiopoietin · Tie2 · VEGF · Prognosis

Abbreviations

HR	hazard ratio
CI	confidence interval
TEM	Tie2-expressing monocyte
TCC	transitional cell carcinoma

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Introduction

Angiogenesis, outgrowth of new vessels from the pre-existing blood vessels, plays an essential role in tumor growth, maintenance and metastasis [1]. This process is regulated by a dynamic balance between angiogenic and anti-angiogenic factors.

Angiopoietin-1 (Ang-1) and Angiopoietin-2 (Ang-2) are angiogenic mediators acting on Tie2 tyrosine kinase receptors [2–4]. Ang-1 promotes interactions between endothelial and neighboring cells thereby is known as a stabilizing factor [3]. Ang-2 is an antagonist of Ang-1 [4]. Shift in the balance of Ang-1 and Ang-2 toward to Ang-2 drives to vessel destabilization which causes either vessel regression or, in presence of vascular endothelial growth factor (VEGF),

vessel formation [5]. Correlation between increased VEGF expression and poor prognosis has been reported in several tumors [6–8]. Increased soluble VEGF levels are present in plasma of renal cell cancer, melanoma and bladder cancer [9–11]. Different expression patterns of Ang-1, Ang-2, VEGF and Tie2 in normal and tumor tissues were found in several tumors such as breast, prostate, colon, lung and ovarian cancer [12–15]. We formerly identified mRNA expression of Tie2 as an independent prognostic indicator of bladder cancer metastasis and survival and Ang-2 as an independent predictor of disease recurrence [16]. Recently, elevated serum levels of Ang-2 were detected in acute myeloid leukemia and lung cancer and were found to correlate with stage progression and patients' survival [17–18].

Tie2 is expressed predominantly on endothelial cells however recently has also been found on surface of monocytes [19]. Through proteolytical cleavage, a 75 kDa extracellular domain of Tie2 (sTie2) appears in human serum [20]. Little is known about the role of this soluble fraction of Tie2.

The aim of the current study was to better understand the role of soluble fraction of these angiogenic factors and to assess the prognostic significance of their serum levels in bladder cancer. Therefore, we analyzed serum VEGF, Ang-1, Ang-2 and sTie2 levels in a large number of bladder cancer patients with a long follow-up period.

Materials and Methods

Clinical Samples

We analyzed frozen serum samples of 117 patients (94 male, 23 female), who underwent surgical treatment of urinary bladder cancer in the Department of Urology of the University Hospital Essen between 1990 and 1994. The mean age of patients was 65.3 years. The criteria for enrollment were histopathological diagnosis of transitional cell carcinoma of the bladder, no history of other tumor, no chemotherapy before surgery, availability of sufficient serum sample and the potential to follow up. The median follow-up period was 24 months with a maximum of 189 months. Serum samples of 64 healthy individuals without history of cancer were used as controls. The ethics committee of Essen University Hospital approved the study protocol. We assessed smoking habits at the time of hospitalization (yes versus no), but data on smoking history or pack-years were not routinely available. Initially all tumors were reclassified according to the 2004 WHO classification of urothelial neoplasm [21]. Preoperative venous blood was centrifuged after collection at 3,000 rpm for 10 min at 4°C. The serum was then separated, aliquoted and stored at −80°C until analysis.

Measurement of Serum VEGF, Ang-1, Ang-2 and Tie2 Levels

Serum levels of VEGF, Ang-1, Ang-2 and Tie2 were quantified by sandwich enzyme-linked immunosorbent assay using DuoSet ELISA kit from R&D Systems (Wiesbaden, Germany), according to the manufacturer's instructions. All samples were examined in duplicate and the mean values were used for statistical analysis.

Statistical Analysis

For statistical analysis, the non-parametric two-sided Wilcoxon rank sum test (Mann–Whitney test) for paired group comparisons was applied. Univariate recurrence-free, metastasis-free and disease-specific survival analysis was done using both Kaplan-Meier log-rank test and univariate Cox analysis. For multiple analysis, the Cox proportional hazards regression model was used. In the survival analysis we considered three different prognostic end points: recurrence, metastasis and bladder cancer related death.

Recurrence-free survival is the interval between surgical treatment and the first local (organ confined) recurrence. Metastasis-free survival refers to the time from surgery to the exploration of distant metastasis. Finally disease-specific survival was defined as the period from surgery until bladder cancer related death. We applied the Spearman's rho test to analyze the correlations between the serum levels of angiogenic factors. In all tests the p value of at least 0.05 was considered to be statistically significant. All statistical analyses were done with SPSS software package, version 14.0.

Results

Relationship of Serum VEGF, Ang-1, Ang-2 and sTie2 with Standard Clinical and Pathological Variables

We did not find any significant correlation between serum Ang-1 and sTie2 levels and patient's sex, age, smoking consumption and primer versus recurrent cancer. On the other hand serum VEGF levels were higher in patients younger than 65 years (p=0.048) and serum Ang-2 levels were higher in women (p=0.041).

The soluble levels of VEGF, Ang-1 and Ang-2 did not correlate with tumor stage and grade. In contrast sTie2 was found to be higher in non-muscle invasive (Ta, T1) cases

Table 1 Patients' characteristics and serum levels of VEGF, Ang-1, Ang-2 and sTie2

	n	VEGF (pg/ml) Median (range)	*P* value	Ang-1 (ng/ml) Median (range)	*p* value	Ang-2 (pg/ml) Median (range)	*p* value	sTie2 (pg/ml) Median (range)	*p* value
Age (36 - 87)									
≤ 65	56	114 (0-546)	0.048	53.6 (25.5-83.5)	0.168	178 (0-1537)	0.667	1584 (284-5594)	0.680
> 65	61	79 (0-663)		53.2 (0 -80.5)		213 (0-5585)		1741 (0-5754)	
Gender									
Male	94	106 (0-663)	0.763	54.3 (0 -83.5)	0.497	183 (0-4238)	0.041	1693 (136-4949)	0.849
Female	23	79 (0-293)		50.2 (30.2-80.5)		407 (0-5585)		1374 (0-5754)	
Stage									
Ta	12	131 (3-293)		49.9 (31.5-61.8)		209 (0-5585)		1707 (98-5754)	
T1	26	102 (0-459)		51.2 (25.5-63.8)		177 (0-3935)		1838 (290-4949)	
T2	25	55 (0-445)		53.3 (39.1-80.5)		169 (0-1292)		1338 (284-5594)	
T3	42	99 (0-663)		53.9 (0 -83.5)		234 (0-1833)		1458 (0-5414)	
T4	12	225 (0-546)		57.7 (30.6-62.6)		162 (0-1537)		2202 (523-4847)	
Non-inv.	38	124 (0-495)	0.202	50.3 (25.5-63.8)	0.130	177 (0-5585)	0.193	1789 (98-5754)	0.025
Invasive	79	87 (0-663)		54.1 (0 -83.5)		213 (0-1833)		1522 (0-5594)	
Grade									
G1	6	111 (20-195)		50.0 (15.2-55.3)		305 (0- 343)		2086 (734-4324)	
G2	42	96 (0-445)		54.5 (27.5-80.5)		155 (0-5585)		1777 (98-5754)	
G3	69	93 (0-663)		53.4 (0 -83.5)		222 (0-1833)		1542 (0-5414)	
Low-grade	48	100 (0-446)	0.784	50.3 (15.2-80.5)	0.955	162 (0-5585)	0.211	1700 (98-5754)	0.115
High-grade	69	93 (0-663)		53.4 (0 -83.5)		222 (0-1833)		1542 (0-5414)	
Primer	57	93 (0-546)	0.278	53.5 (0 -83.5)	0.598	248 (0-5585)	0.405	1702 (0-5754)	0.363
Recurrent	60	105 (0-663)		53.3 (25.5-80.5)		157 (0-1833)		1563 (98-4949)	
Smoking									
yes	56	111 (0-445)	0.338	52.9 (22.2-78.6)	0.989	248 (0-1682)	0.066	1808 (0-5754)	0.132
no	61	87 (0-663)		53.9 (0 -83.5)		162 (0-5585)		1566 (264-5594)	
Control	64	51 (0-452)	0.309	23.4 (0 -74.8)	<0.001	309 (0-6606)	0.016	3881 (625-7018)	0.001
Tumor	117	100 (0-663)		53.3 (0 -83.5)		205 (0-5585)		1684 (0-5754)	

than in muscle invasive tumors (p=0.025; Table 1 and Fig. 1).

Comparison of Serum Levels of Angiogenic Factors Between Tumor Patients and Normal Controls

Angiopoietin-1 levels were found significantly (2-fold) higher in serum samples of tumor patients (p<0.001). In contrast Ang-2 and sTie2 levels were significantly lower in tumor patients than in healthy individuals (p=0.016, p=0.001). Serum VEGF expression levels were higher in tumor patients, however this correlation failed to reach statistical significance (p=0.309; Table 1 and Fig. 1).

Correlation Analysis of Serum VEGF, Ang-1, Ang-2 and sTie2 Expression

Using Spearman-rho test, VEGF levels significantly correlated with both Ang-1, Ang-2 and sTie2 serum levels (p=0.002, p=0.021 and p=0.020 respectively). Furthermore sTie2 correlated with Ang-2 (p=0.018) in contrast to Ang-1 (p=0.323), while Ang-1 serum levels did not correlated with Ang-2 (p=0.300).

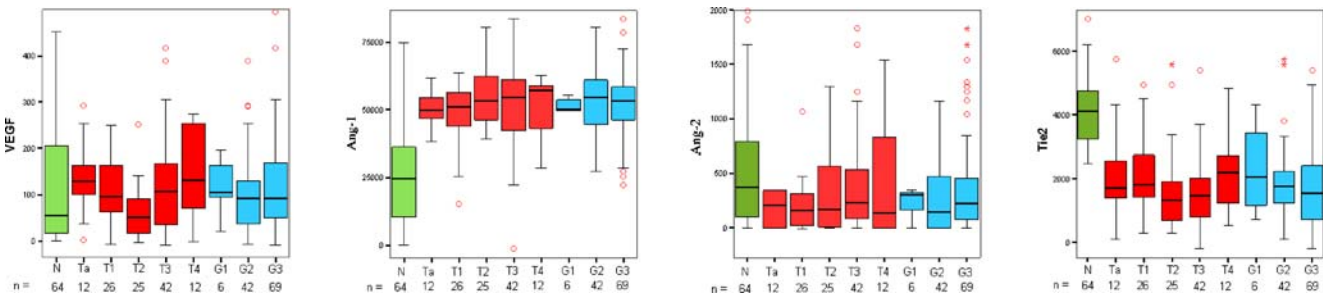

Fig. 1 Serum levels (pg/ml) of angiogen factors (Ang-1, Ang-2, Tie2, and VEGF) by tumor stage and grade in bladder cancer. Boxes represent the 25th to 75th percentiles; horizontal lines show the median values, circles represent the maximum expression (*N* normal control)

Association of Serum Ang-1, Ang-2, VEGF, sTie2 with Disease-Specific Survival, Metastasis-Free Survival and Recurrence-Free Survival

Results of univariate analysis of angiogenic factors and prognostic endpoints (disease specific survival, metastasis-free survival and recurrence-free survival) are listed in Table 2. Figures 2 and 3 show the Kaplan–Meier survival curves. The patients were subdivided into low or high groups for the serum levels of each angiogenic factor using the median value as the cut-off. The cut-off values were as follows: 53.3 ng/ml for Ang-1, 205 pg/ml for Ang-2, 100 pg/ml for VEGF and 1,684 pg/ml for sTie2 (Table 2).

Table 2 Cox univariate analysis

Variables		Disease-Specific survival			Metastasis-free survival			Recurrence-free survival		
	n	HR	95% CI	P	HR	95% CI	P	HR	95% CI	P
Age 65.3 (36 - 87)										
≤ 65	56	ref.			ref.			ref.		
> 65	61	1.25	0.840 – 1.87	0.274	0.98	0.56 – 1.73	0.945	0.86	0.50 – 1.45	0.566
Sex										
Male	94	ref.			ref.			ref.		
Female	23	0.92	0.58 – 1.45	0.706	0.79	0.42 – 1.49	0.458	1.28	0.69 – 2.38	0.442
Stage										
Non-inv.	38	ref.			ref.			ref.		
Invasive	79	3.84	2.34 – 6.30	<0.001	4.09	2.03 – 8.23	<0.001	0.79	0.40 – 1.56	0.493
Grade										
Low-grade	48	ref.			ref.			ref.		
High-grade	69	2.41	1.57 – 3.71	<0.001	2.72	1.48 – 5.02	0.001	0.55	0.28 – 1.10	0.093
Prior recurrence										
Primer	57	ref.			ref.			ref.		
Recurrent	60	1.21	0.80 – 1.82	0.363	0.72	0.40 – 1.28	0.260	0.92	0.55 – 1.56	0.766
Smoking										
no	61	ref.			ref.			ref.		
yes	56	0.86	0.57 – 1.28	0.446	0.74	0.42 – 1.32	0.308	0.78	0.44 – 1.40	0.403
VEGF serum level										
low	60	ref.			ref.			ref.		
high	57	0.70	0.43 – 1.13	0.146	0.69	0.35 – 1.360	0.283	0.96	0.46 – 2.03	0.920
non-inv. cases										
low	15	ref.			ref.			ref.		
high	23	0.17	0.06 – 0.52	0.002	0.48	0.10 – 2.42	0.376	1.96	0.64 – 5.99	0.238
invasive cases										
low	45	ref.			ref.			ref.		
high	34	1.32	0.77 – 2.26	0.322	1.01	0.48 – 2.14	0.982	0.03	0.00 – 112.0	0.392
Ang-1 serum level										
low	59	ref.			ref.			ref.		
high	58	1.14	0.71 – 1.84	0.593	1.78	0.90 – 3.50	0.096	0.68	0.31 – 1.49	0.329
non-inv. cases										
low	22	ref.			ref.			ref.		
high	16	1.05	0.37 – 2.97	0.923	2.04	0.40 – 10.26	0.388	0.41	0.14 – 1.17	0.095
invasive cases										
low	37	ref.			ref.			ref.		
high	42	0.98	0.57 – 1.69	0.936	1.44	0.68 – 3.06	0.346	1.82	0.51 – 6.53	0.357
Ang-2 serum level										
low	60	ref.			ref.			ref.		
high	57	0.83	0.52 – 1.34	0.449	1.16	0.60 – 2.25	0.661	0.48	0.22 – 1.05	0.065
non-inv. cases										
low	21	ref.			ref.			ref.		
high	17	0.38	0.12 – 1.18	0.095	0.65	0.12 – 3.54	0.616	0.74	0.29 – 1.88	0.520
invasive cases										
low	39	ref.			ref.			ref.		
high	40	1.00	0.59 – 1.72	0.993	1.32	0.67 – 2.74	0.456	0.10	0.01 – 0.81	0.006
Tie2 serum level										
low	61	ref.			ref.			ref.		
high	56	1.26	0.76 – 2.08	0.373	1.75	0.87 – 3.52	0.118	0.76	0.33 – 1.61	0.431
non-inv. cases										
low	15	ref.			ref.			ref.		
high	23	1.13	0.38 – 3.38	0.826	1.35	0.25 – 7.38	0.731	1.18	0.41 – 3.35	0.760
invasive cases										
low	46	ref.			ref.			ref.		
high	33	1.67	0.94 – 2.98	0.081	2.47	1.14 – 5.35	0.022	0.25	0.06 – 1.11	0.068

HR hazard ratio, *CI* confidence interval, *Ref.* referent

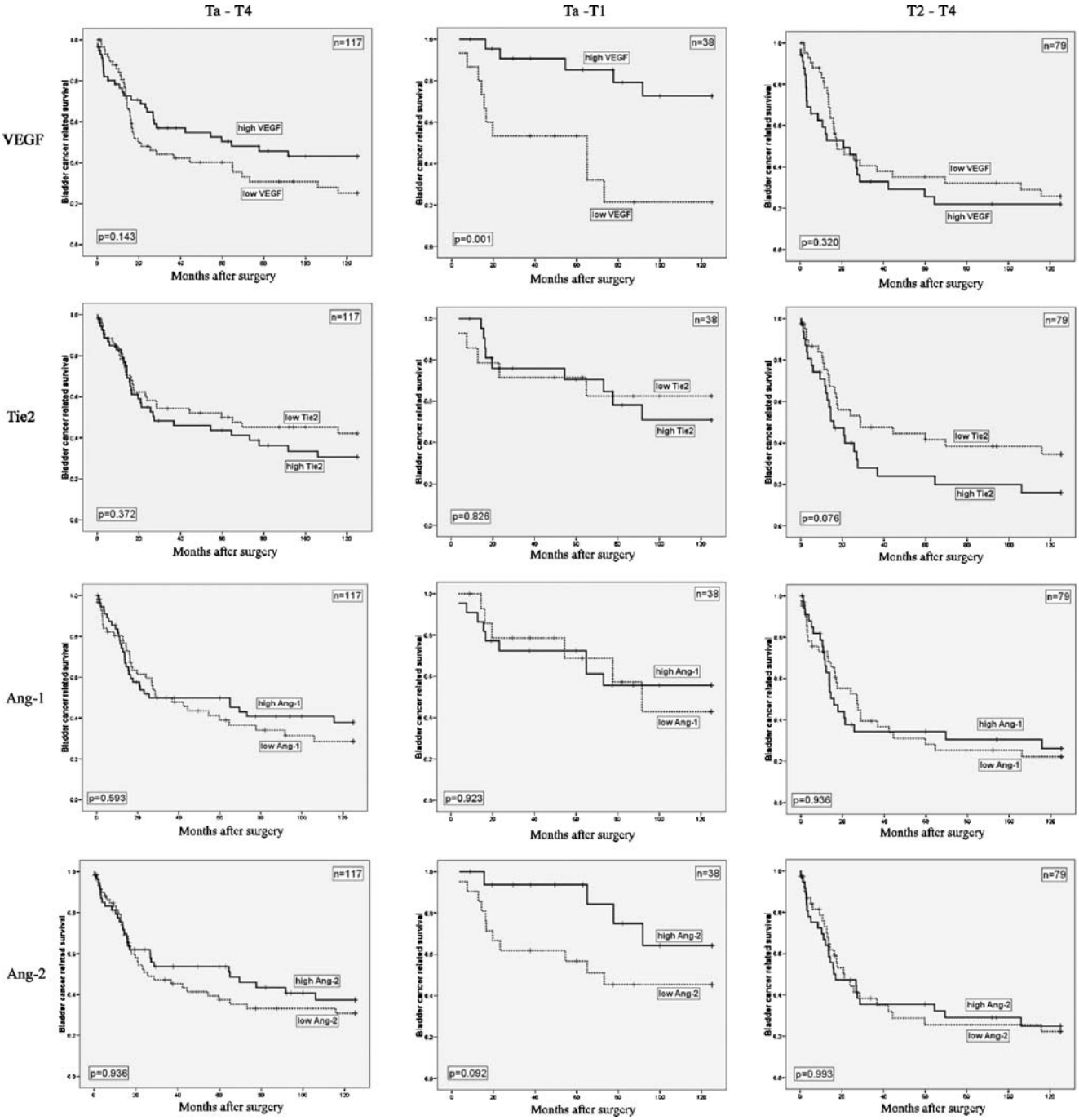

Fig. 2 Kaplan–Meier curves of cancer-specific survival stratified by serum levels of VEGF, Ang-1, Ang-2 and sTie2 in superficial and muscle invasive ($T>1$) bladder tumors. The p values (log rank test) in this figure can slightly differ from the p values (Cox univariate analysis) from that of Table 2

The multivariate Cox model for recurrence-free survival, metastasis-free survival and disease-specific survival included tumor stage, grade and serum levels of analyzed angiogenic factors.

The majority of invasive bladder tumors (68/79) were treated with radical cystectomy. In these cases the probability to observe a local recurrence is limited. Therefore we discuss the recurrence-free survival only in non muscle-invasive cases.

High VEGF serum levels were correlated with longer disease-specific survival in non-invasive (Ta, T1) cases (HR 0.17, 95% CI 0.06–0.52, $p=0.002$; Table 2 and Fig. 2), however multivariate analysis revealed that VEGF is not an independent prognostic factor (Table 3). Regarding Ang-1

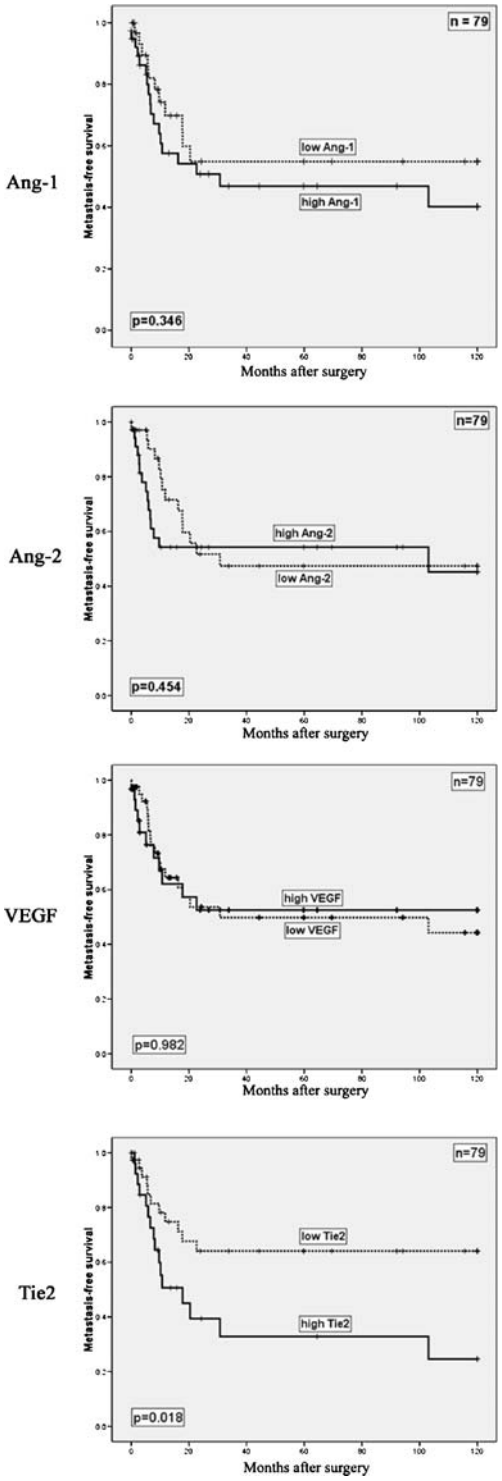

Fig. 3 Kaplan–Meier curves of metastasis-free survival stratified by serum levels of Ang-1, Ang-2, VEGF and sTie2 in muscle invasive ($T>1$) bladder tumors

and Ang-2 serum levels, there was no significant difference in disease-specific and metastasis-free survival and in case of Ang-1 also not in recurrence-free survival (Figs. 2 and 3). High serum levels of Tie2 were correlated with shorter metastasis-free survival both in univariate (HR 2.466, CI 95% 1.14–5.35, $p=0.022$) and in multivariate analysis (HR 2.393, 95% CI 1.17–4.88, $p=0.016$) thus proved as an independent risk factor for metastasis (Fig. 3). Furthermore high Tie2 serum levels tended to correlate with shorter disease-free survival in muscle invasive cases both in univariate and multivariate analysis however this failed to reach the prognostic significance ($p=0.081$ in univariate and $p=0.069$ in multivariate analysis; Fig. 2).

The most powerful predictor of distance metastasis and bladder cancer related death was tumor stage (HR 4.09, 95% CI 2.03–8.23, $p<0.001$ and HR 3.84, 95% CI 2.34–6.30, $p<0.001$ respectively) and tumor grade (HR 2.72, 95% CI 1.48–5.02, $p=0.001$ and HR 2.41, 95% CI 1.57–3.71, $p<0.001$ respectively).

Discussion

Our present report demonstrates a significant elevation of Ang-1, a twofold, although not significant, elevation of VEGF, a clear decrease of sTie2 and a mild decrease of Ang-2 levels in serum samples of bladder cancer patients. Furthermore, we identified for the first time soluble serum level of the extracellular domain of the receptor tyrosine kinase (Tie2) as an independent predictor of disease prognosis.

Angiogenesis is a complex dynamic process controlled by vessel formation and regression. It is essential for tumor growth, maintenance and metastasis and regulated by a dynamic interaction between pro- and anti-angiogenic factors. Angiopoietins represent a family which members bind with equal affinity to the extracellular domain of Tie2, a tyrosine kinase receptor, expressed mainly on endothelial cells. Ang-1, due to the activation of Tie2, promotes maturation of endothelial cells and stabilization of newly formed blood vessels via assembling of peri-endothelial cells such as pericytes or smooth muscle cells into the vascular wall [22]. Ang-2 acts as an antagonist on Tie2 receptor, provokes destabilization of blood vessels and sensitizes endothelial cells to VEGF [23].

Conflicting results were reported regarding VEGF expression in bladder cancer. While, in some studies, a higher expression of VEGF was documented in low-stage superficial compared to high-stage muscle invasive bladder cancer, others reported the opposite [24–26]. We formerly detected markedly higher VEGF expression in superficial bladder cancer compared to muscle invasive TCC in a large number of biopsies (Szarvas et al., manuscript submitted for publication). This suggests a strong proangiogenic stimulus already in superficial stages (Ta/T1) of bladder cancer. This is in line with the findings of Oliveira-Ferrer et al. [27] demonstrating epithelial down-regulation of a carcinoem-

Table 3 Multivariate Cox regression analyses of histopathological parameters and serum levels of angiogenic factors on disease-specific, metastasis-free and recurrence-free survival in bladder cancer patients

Variables		Disease-specific survival			Metastasis-free survival			Recurrence-free survival		
		HR	95% CI	*p*	HR	95% CI	*p*	HR	95% CI	*p*
Stage	(Ta, T1)	Ref.			Ref.			Ref.		
	(T2-T4)	2.222	1.177–4.193	0.014	2.922	1.102–7.746	0.031	1.571	0.604–4.086	0.355
Grade	(G1-G2)	Ref.			Ref.			Ref.		
	(G3)	0.112	0.901–2.709	0.112	1.709	0.761–3.838	0.194	0.902	0.376–2.161	0.816
VEGF serum level	low	Ref.			Ref.			Ref.		
	high	0.921	0.561–1.511	0.744	0.959	0.481–1.912	0.904	1.083	0.454–2.585	0.857
Stage	(Ta, T1)	Ref.			Ref.			Ref.		
	(T2-T4)	2.159	1.158–4.025	0.015	2.512	0.962–6.559	0.060	1.480	0.640–3.422	0.359
Grade	(G1-G2)	Ref.			Ref.			Ref.		
	(G3)	1.540	0.890–2.665	0.123	1.866	0.837–4.157	0.127	0.825	0.349–1.950	0.661
Ang-1 serum level	low	Ref.			Ref.			Ref.		
	high	1.038	0.640–1.683	0.880	1.675	0.841–3.333	0.142	0.661	0.297–1.473	0.311
Stage	(Ta, T1)	Ref.			Ref.			Ref.		
	(T2-T4)	2.213	1.188–4.123	0.012	2.892	1.101–7.594	0.031	1.758	0.744–4.154	0.198
Grade	(G1-G2)	Ref.			Ref.			Ref.		
	(G3)	1.589	0.915–2.759	0.100	1.691	0.755–3.787	0.201	0.799	0.382–2.099	0.799
Ang-2 serum level	low	Ref.			Ref.			Ref.		
	high	0.794	0.491–1.285	0.347	1.114	0.573–2.167	0.750	0.448	0.203–0.989	0.047
Stage	(Ta, T1)	Ref.			Ref.			Ref.		
	(T2-T4)	2.361	1.231–4.528	0.010	2.947	1.095–7.932	0.032	0.724	0.594–3.488	0.420
Grade	(G1-G2)	Ref.			Ref.			Ref.		
	(G3)	1.632	0.918–2.901	0.095	2.039	0.869–4.786	0.102	1.439	0.291–1.803	0.488
Tie-2 serum level	low	Ref.			Ref.			Ref.		
	high	1.615	0.963–2.709	0.069	2.393	1.174–4.880	0.016	0.666	0.292–1.517	0.333
Stage	(Ta, T1)	Ref.			Ref.			Ref.		
	(T2-T4)	2.181	1.088–4.373	0.028	2.527	0.908–7.030	0.076	1.437	0.474–4.358	0.522
Grade	(G1–2)	Ref.			Ref.			Ref.		
	(G3)	1.661	0.934–2.953	0.084	2.163	0.923–5.070	0.076	0.638	0.242–1.680	0.363
VEGF serum level	Low	Ref.			Ref.			Ref.		
	High	0.959	0.545–1.685	0.884	0.928	0.429–2.004	0.849	0.903	0.315–2.588	0.850
Ang-1 serum level	Low	Ref.			Ref.			Ref.		
	High	1.069	0.630–1.812	0.806	1.602	0.711–3.329	0.207	0.891	0.367–2.162	0.798
Ang-2 serum level	Low	Ref.			Ref.			Ref.		
	High	0.703	0.417–1.185	0.186	0.897	0.440–1.828	0.765	0.344	0.137–0.867	0.024
Tie-2 serum level	Low	Ref.			Ref.			Ref.		
	High	1.721	1.004–2.949	0.048	2.417	1.149–5.082	0.020	0.611	0.262–1.422	0.253

bryonic cell-adhesion molecule CEACAM1 induced angiogenesis via up-regulation of VEGF in superficial bladder cancer. In accordance, we detected significantly higher serum VEGF levels in superficial cases than in muscle invasive ones (Table 1). Furthermore, others demonstrated in a functional (human umbilical vein endothelial cells – HUVEC) assay that sera from patients with superficial (Ta) and well differentiated tumors show a significantly higher angiogenic activity than that of patients with invasive and poorly differentiated tumors. This significantly correlated with higher serum VEGF levels [28]. Taking together, superficial bladder tumors express a strong angiogenic signal characterized by high tissue and high serum levels of VEGF. This may be related to the fact that superficial bladder carcinomas are not vascularized and therefore need to activate vessel remodeling/angiogenesis. In contrast, muscle-invasive bladder cancers are vascularized which can reduce hypoxia, the main driving force of angiogenesis. This may explain the lower VEGF levels we and others found both in tissue and serum samples of patients with advanced bladder cancer. The down-regulation of VEGF may present an important step in the progression of bladder cancer. Beecken et al. [28] observed that patients with low serum angiogenic activity (and VEGF serum levels) have a higher risk of distant metastasis and shorter patients' survival. This is in accordance with our present finding, that high VEGF serum level is a favourable prognostic factor in superficial cases of bladder cancer.

Both Ang-1 and Ang-2 expression was found to be elevated in various human tumors [29]. Previously, in accordance with Quentin et al. we detected strongly decreased Ang-1 and a slightly increased Ang-2 mRNA expression in bladder TCC [26]. In contrast others found no difference in the abundance of the two angiopoietins between normal and tumor specimens by immunohistochemical analysis. They found correlation between Ang-2, but not Ang-1 expression and tumor stage and grade, and concluded that Ang-2 might provide prognostic information in bladder cancer [30].

High soluble Ang-2 levels found to be an unfavourable prognostic factor of metastasis and survival in acute myeloid leukemia and lung cancer [31–32]. We detected a significant correlation between Ang-2 serum levels and disease recurrence in invasive bladder cancer, however this observation suggest caution since these cases are treated by radical cystectomy as far as patinets' condition permits it (Table 2). Therefore this group of patients is strongly selected. In order to avoid the misinterpretation of data we restricted our observations of recurrence solely to superficial bladder cancer cases.

Tie2 is expressed in endothelial cells, mainly in a membrane-bound form, however recent publications described the Tie2-expression in a subset of monocytes (TEMs). TEMs were found to represent the main monocyte fraction in tumor and thought to play a driving role in tumor angiogenesis [33]. The extracellular domain of Tie2 is proteolytically cleaved, resulting in a 75-kDa soluble receptor fragment (sTie2) [34]. Although the role of sTie2 is unclear, serum levels of sTie2 in renal cell carcinoma were correlated with higher tumor stage and mortality, suggesting its importance in tumor angiogenesis [35]. In the present study we demonstrate that high sTie2 is an independent prognostic factor of metastasis (HR 2.39, 95% CI 1.17–4.88, $p=0.016$) and a borderline significant predictor of cancer related survival (HR 1.62, 95% CI 0.96–2.71, $p=0.069$) in invasive bladder cancer. Based on our former notion that higher Tie2 mRNA expression is a favourable prognostic factor in bladder cancer, we hypothesize that Tie2 shedding plays a causal role in the activation of angiogenesis through inhibition of stabilizing effect of Ang1/Tie2 interaction. This is further supported by the current notion that VEGF, the best known angiogenic activator, induces Tie2 shedding via PI3K/Akt dependent pathway [36]. The significant correlation between serum levels of VEGF and sTie2 ($p=0.02$) in patients we observed also supports this interpretation. On the other hand in a rat cutaneous tumor model the use of soluble Tie2 systematically blocked tumor vessel proliferation and inhibited the growth of primary tumors and metastases [37]. However, the model used in this study does not represent the whole shedding process since it concentrates solely on the effect of elevated soluble Tie2, and disregards the consequences caused by the absence of extracellular Tie2 domain. Our results suggest that the prognostically unfavourable vessel destabilizing effect of Tie2 shedding is dominant over the assumed favourable antiproliferative effect of higher sTie2 levels.

The mechanisms responsible for the significant higher sTie2 levels in control samples are unclear and should therefore be the subject of further research. We presume different origin of sTie2 in healthy individuals and in bladder cancer patients.

Conclusions

To our knowledge this is the first study analyzing the serum levels of Ang-1, Ang-2 and the soluble extracellular domain of their receptor; Tie2 (sTie2) in urinary bladder cancer. We found significantly elevated Ang-1 and significantly decreased sTie2 and Ang-2 levels in sera of bladder cancer patients. Soluble Tie2 may serve as a diagnostic tool for selecting high risk group of patients independent from tumor stage and grade.

Acknowledgement We thank Jacqueline Wittschier for excellent technical assistance. Grant support from the German National Federal Ministry of Education and Research (0313659B) is also acknowledged.

References

1. Weidner N, Semple JP, Welch WR, Folkman J (1991) Tumor angiogenesisand metastasis correlation in invasive breast carcinoma. N Engl J Med 324:1–8
2. Davis S, Aldrich TH, Jones PF, Acheson A, Compton DL, Jain V, Ryan TE, Bruno J, Radziejewski C, Maisonpierre PC, Yancopoulos GD (1996) Isolation of angiopoietin-1, a ligand for the TIE2 receptor, by secretion-trap expression cloning. Cell 87:1161–1169
3. Papapetropoulos A, Garcia-Cardena G, Dengler TJ, Maisonpierre PC, Yancopoulos GD, Sessa WC (1999) Direct actions of angiopoietin-1 on human endothelium: evidence for network stabilization, cell survival, and interaction with other angiogenic growth factors. Lab Invest 79:213–223
4. Maisonpierre PC, Suri C, Jones PF, Bartunkova S, Wiegand SJ, Radziejewski C, Compton D, McClain J, Aldrich TH, Papadopoulos N, Daly TJ, Davis S, Sato TN, Yancopoulos GD (1997) Angiopoietin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis. Science 277:55–60
5. Holash J, Maisonpierre PC, Compton D, Boland P, Alexander CR, Zagzag D, Yancopoulos GD, Wiegand SJ (1999) Vessel cooption, regression, and growth in tumors mediated by angiopoietins and VEGF. Science 284:1994–1998
6. Volm M, Koomagi R, Mattern J (1997) Prognostic value of vascular endothelial growth factor and its receptor flt-1 in squamous cell lung cancer. Int J Cancer 74:64–68
7. Inoue K, Ozeki Y, Suganuma T, Sugiura Y, Tanaka S (1997) Vascular endothelial growth factor expression in primary esophageal squamous cell carcinoma. Cancer 79:206–213

8. Maeda K, Chung Y, Takatsuka S, Ogawa Y, Sawada T, Yamashita Y et al (1995) Tumor angiogenesis as a predictor of recurrence in gastric carcinoma. J Clin Oncol 13:477–481
9. Dosquet C, Coudert MC, Lepage E, Cabane J, Richard F (1997) Are angiogenic factors, cytokines, and soluble adhesion molecules prognostic factors in patients with renal cell carcinoma? Clin Cancer Res 3:2451–2458
10. Tas F, Duranyildiz D, Oguz H, Camlica H, Yasasever V, Topuz E (2006) Circulating serum levels of angiogenic factors and vascular endothelial growth factor receptors 1 and 2 in melanoma patients. Melanoma Res 16:405–411
11. Bernardini S, Fauconnet S, Chabannes E, Henry PC, Adessi G, Bittard H (2001) Serum levels of vascular endothelial growth factor as a prognostic factor in bladder cancer. J Urol 166:1275–1279
12. Sfiligoi C, de Luca A, Cascone I, Sorbello V, Fuso L, Ponzone R, Biglia N, Audero E, Arisio R, Bussolino F, Sismondi P, De Bortoli M (2003) Angiopoietin-2 expression in breast cancer correlates with lymph node invasion and short survival. Int J Cancer 103:466–474
13. Wurmbach JH, Hammerer P, Sevinc S, Huland H, Ergun S (2000) The expression of angiopoietins and their receptor Tie-2 in human prostate carcinoma. Anticancer Res 20:5217–5220
14. Wong MP, Chan SY, Fu KH, Leung SY, Cheung N, Yuen ST, Chung LP (2000) The angiopoietins, tie2 and vascular endothelial growth factor are differentially expressed in the transformation of normal lung to non-small cell lung carcinomas. Lung Cancer 29:11–22
15. Hata K, Nakayama K, Fujiwaki R, Katabuchi H, Okamura H, Miyazaki K (2004) Expression of the angopoietin-1, angopoietin-2, Tie2, and vascular endothelial growth factor gene in epithelial ovarian cancer. Gynecol Oncol 93:215–222
16. Szarvas T, Jäger T, Tötsch M, vom Dorp F, Kempkensteffen C, Kovalszky I, Romics I, Ergün S, Rübben H (2008) Angiogenic switch of angiopietins-Tie2-system and its prognostic value in bladder cancer. Clin Cancer Res (in press)
17. Liersch R, Schliemann C, Bieker R, Hintelmann H, Buechner T, Berdel WE, Mesters RM (2008) Expression of VEGF-C and its receptor VEGFR-3 in the bone marrow of patients with acute myeloid leukaemia. Leuk Res 32:954–961
18. Park JH, Park KJ, Kim YS, Sheen SS, Lee KS, Lee HN, Oh YJ, Hwang SC (2007) Serum angiopoietin-2 as a clinical marker for lung cancer. Chest 132:200–206
19. Lewis CE, De Palma M, Naldini L (2007) Tie2-expressing monocytes and tumor angiogenesis: regulation by hypoxia and angiopoietin-2. Cancer Res 67:8429–8432
20. Reusch P, Barleon B, Weindel K, Martiny-Baron G, Gödde A, Siemeister G, Marmé D (2001) Identification of a soluble form of the angiopoietin receptor TIE-2 released from endothelial cells and present in human blood. Angiogenesis 4:123–131
21. Eble JN, Epstein JI, Sesterhenn I, World Health Organization classification of tumors (2004) Pathology and genetics of tumors of the urinary system and male genital organs. IARCC, Lyon, pp 89–158
22. Suri C, Jones PF, Patan S, Bartunkova S, Maisonpierre PC, Davis S, Sato TN, Yancopoulos GD (1996) Requisite role of angiopoietin-1, a ligand for the TIE2 receptor, during embryonic angiogenesis. Cell 87:1171–1180
23. Holash J, Wiegand SJ, Yancopoulos GD (1999) New model of tumor angiogenesis: dynamic balance between vessel regression and growth mediated by angiopoietins and VEGF. Oncogene 18:5356–5362
24. Crew JP, Fuggle S, Bicknell R, Cranston DW, de Benedetti A, Harris AL (2000) Eukaryotic initiation factor-4E in superficial and muscle invasive bladder cancer and its correlation with vascular endothelial growth factor expression and tumour progression. Br J Cancer 82:161–166
25. O'Brien T, Cranston D, Fuggle S, Bicknell R, Harris AL (1995) Different angiogenic pathways characterize superficial and invasive bladder cancer. Cancer Res 55:510–513
26. Quentin T, Schlott T, Korabiowska M, Kathei N, Zoller G, Glaser F, Kunze E (2004) Alteration of the vascular endothelial growth factor and angiopoietins-1 and -2 pathways in transitional cell carcinomas of the urinary bladder associated with tumor progression. Anticancer Res 24:2745–2756
27. Oliveira-Ferrer L, Tilki D, Ziegeler G, Hauschild J, Loges S, Irmak S, Kilic E, Huland H, Friedrich M, Ergün S (2004) Dual role of carcinoembryonic antigen-related cell adhesion molecule 1 in angiogenesis and invasion of human urinary bladder cancer. Cancer Res 64:8932–8938
28. Beecken WD, Engl T, Hofmann J, Jonas D, Blaheta R (2005) Clinical relevance of serum angiogenic activity in patients with transitional cell carcinoma of the bladder. J Cell Mol Med 9:655–661
29. Tait CR, Jones PF (2004) Angiopoietins in tumours: the angiogenic switch. J Pathol 204:1–10
30. Oka N, Yamamoto Y, Takahashi M, Nishitani M, Kanayama HO, Kagawa S (2005) Expression of angiopoietin-1 and -2, and its clinical significance in human bladder cancer. BJU Int 95:660–663
31. Schliemann C, Bieker R, Thoennissen N, Gerss J, Liersch R, Kessler T, Büchner T, Berdel WE, Mesters RM (2007) Circulating angiopoietin-2 is a strong prognostic factor in acute myeloid leukemia. Leukemia 21:1901–1906
32. Park JH, Park KJ, Kim YS, Sheen SS, Lee KS, Lee HN, Oh YJ, Hwang SC (2007) Serum angiopoietin-2 as a clinical marker for lung cancer. Chest 132:200–206
33. Lewis CE, De Palma M, Naldini L (2007) Tie2-expressing monocytes and tumor angiogenesis: regulation by hypoxia and angiopoietin-2. Cancer Res 67:8429–8432
34. Reusch P, Barleon B, Weindel K, Martiny-Baron G, Gödde A, Siemeister G, Marmé D (2001) Identification of a soluble form of the angiopoietin receptor TIE-2 released from endothelial cells and present in human blood. Angiogenesis 4:123–131
35. Harris AL, Reusch P, Barleon B, Hang C, Dobbs N, Marme D (2001) Soluble Tie2 and Flt1 extracellular domains in serum of patients with renal cancer and response to antiangiogenic therapy. Clin Cancer Res 7:1992–1997
36. Findley CM, Cudmore MJ, Ahmed A, Kontos CD (2007) VEGF induces Tie2 shedding via a phosphoinositide 3-kinase/Akt dependent pathway to modulate Tie2 signaling. Arterioscler Thromb Vasc Biol 27:2619–2626
37. Lin P, Polverini P, Dewhirst M, Shan S, Rao PS, Peters K (1997) Inhibition of tumor angiogenesis using a soluble receptor establishes a role for Tie2 in pathologic vascular growth. J Clin Invest 100:2072–2078