REVIEW PAPER   UROLOGICAL ONCOLOGY

Role of angiogenesis in urothelial bladder carcinoma

Łukasz Piotr Fus, Barbara Górnicka

Department of Pathology, Medical University of Warsaw, Warsaw, Poland

Corresponding author
Łukasz Piotr Fus
Medical University
of Warsaw
Department of Pathology
7, Pawińskiego Street
02-106 Warsaw, Poland
phone: +48 691 089 000,
+48 22 599 16 95
lukasz.fus@wum.edu.pl

Introduction Bladder cancer is the most common urinary tract malignancy in western countries. In recent years, extensive research has suggested that angiogenesis plays an important role in bladder cancer biology, contributing to tumor growth and progression.
Material and methods In this review, we discuss general mechanisms of angiogenesis and highlight the influence of pro- and anti-angiogenic factors, and cancer stem cells on bladder cancer biology, their relation to disease progression, and potential use in novel targeted therapies.
Results Expression of a number of proangiogenic factors, including HIF-1, VEGF, bFGF, IL-8 and MMPs, as well as anti-angiogenic factor TSP-1, was found to be altered in bladder tumors. Involvement of cancer stem cells in bladder cancer development was also proposed.
Conclusions High expression of most pro-angiogenic factors correlated with disease progression and shorter patient survival, but discrepancies between studies urge us to continue evaluating the significance of angiogenesis in bladder cancer.

Key Words: urothelial carcinoma ‹› bladder cancer ‹› angiogenesis ‹› angiogenic factors ‹› biomarkers ‹› cancer stem cells

INTRODUCTION

Angiogenesis – the formation of new capillaries from pre-existing blood vessels – is vital for tumor growth and metastasis. It is a complex process, dependent on multiple factors and involves signaling pathways regulating several aspects of cell biology. In recent years, a growing number of researchers investigated the role of angiogenesis in urinary bladder malignancies. Bladder cancer is the most common urinary tract cancer and the fourth-most common malignancy in men in developed countries. In Europe and North America, over 90% of bladder cancers are urothelial bladder carcinomas (UBC). The majority of patients with UBC initially present with a low-grade tumor confined to the mucosa with a high recurrence rate after transurethral resection. Up to one third of these non-muscle invasive cancers (NMIBC) demonstrate disease progression to more invasive or higher-grade tumors. One of the steps in cancer progression is angiogenesis and our knowledge regarding its significance in UBC is constantly expanding. Bladder tumors were found to produce high levels of factors involved in stimulation of angiogenesis, including vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), interleukin–8 (IL-8) and matrix metalloproteinases (MMPs). Tissue, serum or urine levels of these molecules often correspond with tumor stage and clinical outcome, and there have been reports of potential therapeutic use of angiogenesis inhibitors in UBC. In this review, we highlight the key information regarding mechanisms behind angiogenesis in UBC and its clinical significance.

Angiogenesis – general information

Angiogenesis is an essential part of many physiological processes, such as tissue adaptation to hypoxia or regeneration. It is also of crucial importance for cancer growth and survival of neoplastic cells. Angiogenesis is a strictly controlled, multi-step, dynamic process regulated by a plethora of growth factors, cytokines, enzymes, extracellular matrix (ECM) proteins and other mediators. In the physiological state, tissues maintain

a steady balance between endogenous angiogenesis inducers (pro-angiogenic factors) and inhibitors (anti-angiogenic factors). In cancers, when the balance is disrupted and shifted towards pro-angiogenic factors, the so-called angiogenic switch is activated and new vasculature formation starts. The best-known mechanism of tumor angiogenesis is capillary sprouting – the development of new buds from pre-existing tissue capillaries. In this process, a blood vessel initially dilates, becomes more permeable and the basement membrane is partly degraded at the site where the pro-angiogenic stimulus is strongest. Then, endothelial cells (EC) migrate into surrounding tissues forming a bud with a slit-like lumen maintaining a connection with the mother vessel. Finally, the newly formed capillary is covered by vessel supporting cells – pericytes. Recent studies identified additional mechanisms involved in tumor vascularization, namely vessel co-option, intussusceptive microvascular growth (IMG) and postnatal vasculogenesis. Vessel co-option is based on the incorporation of existing vessels into the expanding tumor, which later results in separation of that vessel from host tissue. In IMG, the vessel lumen is divided by formation of transluminal endothelial bridges, which further develop into pillars of connective tissues partitioning the lumen and eventually dividing the vessel in two. An important feature of IMG is its independence from EC proliferation; therefore anti-angiogenic therapies targeting mitogenic activity of ECs may be useless against tumors demonstrating this type of vascularization [1]. It has long been believed that vasculogenesis – development of new blood vessels from mesenchymal progenitors – was restricted to embryonic development, but the discovery of circulating and bone-marrow derived endothelial progenitor cells (EPC) that can be mobilized by tumor cells and incorporated into developing tumor capillary network gave birth to the concept of postnatal vasculogenesis. Although detected in only a fraction of tumor vessels, EPCs play an important role in the activation of ECs and production of pro-angiogenic factors [2]. The described mechanisms of angiogenesis and tumor vascularization are often intermingled, occurring simultaneously. Tumor vasculature can be characterized by distinct structural and functional features, such as a lack of arterial or venous specification, hyperpermeability, lack of pericyte coverage and incorporation of cancer cells into the vessel wall. A complex network of tumor vessels with tortuous shapes, uneven diameters, abnormal shunts and blind-ends forms a disorganized labyrinth with chaotic, inefficient blood flow. Therefore, ironically, growing tumors are often hypoxic despite rich vascularization. Hypoxia causes the up-regulation of hypoxia inducible factors (HIFs), which, in turn, can induce expression of genes with adaptive functions.

Hypoxia inducible factors

HIFs are a family of three transcription factors (HIF-1, -2 and -3) that in the setting of hypoxic stress directly activate expression of numerous pro-angiogenic inducers, as well as suppress the expression of anti-angiogenic factors. The best-described factor is HIF-1, a heterodimeric transcription factor composed of two subunits: HIF-1α and HIF-1β. HIF-1β expression is continuous and remains at steady levels, while in normoxic conditions the HIF-1α subunit undergoes degradation. However, when oxygen concentration drops HIF-1α becomes hydroxylated, preventing it from being degraded and resulting in its accumulation in the cell. In hypoxic cells HIF-1 regulates the expression of pro-angiogenic factors, including VEGF, angiopoietin 1 and 2, platelet-derived growth factor and placental growth factor, as well as expression of enzymes such as MMPs. Among numerous genes regulated by HIF-1, VEGF and its receptors play a central role in the process of angiogenesis.

In vitro studies of UBC cell lines revealed that in hypoxic conditions HIF-1α protein levels are elevated and correspond to increased VEGF expression. Overexpression of HIF-1α was demonstrated in human UBC tissues and it correlated with tumor grade, disease progression and recurrence, and was associated with poor overall survival [3, 4, 5]. Close relations between HIF-1 immunoreactivity, proliferation index, VEGF expression and microvessel density (MVD) were also reported [4]. These results suggest that HIF-1α may serve as a prognostic marker and target in future UBC therapies. Interestingly, HIF-1α can be regulated by mTOR kinase, which provides the possibility of targeting hypoxia signaling pathways with mTOR therapeutics already in use or in clinical trials [6].

Vascular endothelial growth factors

The VEGF family of genes contains several members, including VEGF-A, which plays a major role in angiogenesis, VEGF-B, with a possible role in ECM degradation and migration of ECs, VEGF-C, which is involved primarily in regulation of lymphangiogenesis, and others. VEGFs bind to three types of receptors (VEGFR1-3) containing tyrosine kinase activity. VEGF-A interacts mainly with VEGFR2 expressed on ECs as well as on bone marrow-derived EPC. Binding to a receptor starts cellular signaling pathways resulting in increased permeability of blood vessels, proliferation and migration of ECs, recruitment of EPCs and maintenance of newly formed vasculature.

VEGF overexpression can be detected in the majority of cancers, including bladder cancer. Most researchers agree that levels of tissue VEGF-A correlate with

UBC grade [7, 8], but there are conflicting reports regarding its relation to tumor progression [9, 10, 11]. The prognostic value of tissue VEGF-A expression in UBC also remains unclear: high levels of VEGF-A were found to be associated with worse survival and higher recurrence rates [7, 9, 12], but a number of reports present contradicting results [10, 11]. Serum VEGF-A levels in patients with UBC showed correlation with tumor grade, stage, vascular invasion and the presence of carcinoma *in situ,* and VEGF-A values exceeding 400 pg/ml were highly predictive of metastatic disease [13]. High levels of urine VEGF-A were found to correlate with recurrence in NMIBC [14].

In vitro studies showed that angiogenesis inhibition effectively decreased proliferation and invasion of UBC, leading to further investigation of angiogenesis-targeted agents. Treatment of advanced UBC with bevacizumab (VEGF antibody), and more recently ramucirumab (VEGFR2 antibody), in combination with chemotherapy showed promising results in phase II clinical trials, and are currently in phase III. There are multiple ongoing phase II studies with other agents targeting VEGF receptors, including sunitinib, sorafenib and pazopanib [15].

Fibroblast growth factors

FGFs are growth and differentiation factors, which play fundamental roles in embryonic development, tissue regeneration, angiogenesis and neoplastic transformation. The FGF family is comprised of over 20 ligands that bind to four receptors (FGFR 1-4). In the context of angiogenesis, the most extensively studied are acidic FGF (aFGF) and basic FGF (bFGF). Produced by stromal and endothelial cells, FGFs are localized mainly in the ECM where they form complexes with proteoglycans to avoid degradation. During tumor angiogenesis enzymes, such as proteinases, can mobilize FGFs from the ECM. On release, FGFs bind to receptors with tyrosine kinase activity that transmit a signal to various cytoplasmatic signaling pathways implicated in proliferation, migration and survival of ECs, as well as in formation of a favorable microenvironment for tumor vascularization by increasing expression of other pro-angiogenic factors.

Overexpression of bFGF in UBC is associated with features of aggressive cancer, such as muscle invasion, high tumor grade, chemotherapy resistance, high recurrence rate and poor prognosis [16, 17]. The level of bFGF mRNA in UBC biopsies was found to correlate with MVD as well [18]. In contrast to tissue expression, serum bFGF levels are elevated in patients with NMIBC and low-grade UBC [19]. Compared to the normal population, the level of bFGF in UBC

patients' urine is increased, correlating with tumor grade, stage and tumor recurrence [20].

The presence of activating mutations in the FGFR3 receptor gene in 50–70% of NMIBC strongly suggest its involvement in UBC biology [21], but only a few studies evaluated the link between FGFR3 and tumor angiogenesis. The occurrence of an FGFR3 mutation is related to higher vascularization of the tumor, which suggests that activated FGFR3 acts as a stimulating factor for angiogenesis [22]. FGFR3 mutations are associated with low stage and low-grade tumors and the prevalence of expression decreases with increasing depth of tumor invasion and higher grade [23]. Both the FGFR3 activating mutations and overexpression of wild-type FGFR3 receptor were found to be responsible for favorable outcomes in UBC, as patients had significantly lower risk of tumor recurrence, progression and death [22, 23, 24]. Interestingly, recent data shows that detection of FGFR3 mutation in the urine of patients with low-grade UBC actually indicates tumor recurrence and could be employed as a recurrence-predicting marker [25].

Currently three drug families targeting FGF/FGFR are under development, including tyrosine-kinase inhibitors, monoclonal anti-FGFR antibodies and FGF-trapping molecules. Pan-FGFR tyrosine kinase inhibitors showed promising results in phase I studies. A phase II trial investigating dovitinib (small-molecule FGFR1,3 and VEGFR1,2,3 inhibitor) resulted in a very poor response to treatment in patients with or without FGFR3 mutations [15].

Interleukin-8

Il-8 is a proinflammatory cytokine initially described as a leukocyte chemoattractant, but subsequently identified as a potent mitogenic, angiogenic and growth factor. In various types of cancers increased expression of IL-8 was detected in neoplastic cells, ECs, infiltrating neutrophils and tumor associated macrophages, suggesting its role as a major regulatory factor within the tumor microenvironment. The biological effects of IL-8 are mediated through interaction with two surface receptors, CXCR1 and CXCR2. Activation of IL-8 signaling leads to proliferation, survival and increased motility of ECs, cancer cells, and infiltrating inflammatory cells. In cancers IL-8 was shown to induce tumor angiogenesis, tumorigenicity, lymph node involvement and formation of metastasis, especially at the early phase of tumor growth [26].

Expression of IL-8 in UBC cells is associated with formation of tumor, high metastatic potential, increased MVD and high tumor stage. IL-8 effects on bladder cancer biology can be attributed, at least partially, to regulation of MMPs levels, but IL-8

activity appears to be independent of VEGF or bFGF [26, 27]. Expression of IL-8 is directly regulated by transcription factor NF- κB. Blocking of NF- κB was shown to reduce constitutive expression of VEGF and IL-8, and subsequently to suppress angiogenesis, invasion and metastasis in prostate cancer models [28]. *In vitro* studies of transitional cancer cell lines showed that in less aggressive cancers levels of IL-8 and NF- κB are low, and their expression can be induced by hypoxia and acidosis. In contrast, high-grade cancer cells constitutively express high IL-8 and NF- κB levels, and stressful conditions do not further increase those levels. Furthermore, blocking NF- κB prevents the induction of IL-8, which results in inhibition of tumor growth, angiogenesis and metastasis in human urothelial cancer xenograft models [29].

Matrix metalloproteinases

MMPs are a family of at least 26 proteolytic enzymes, responsible for tissue remodeling and degradation of ECM structural components creating space for cell migration. By cleaving ECM proteins MMPs also regulate the activity of enzymes, growth factors, cytokines and other molecules. As a consequence, MMPs influence several physiological and pathological processes, including angiogenesis. The activities of MMPs are tightly regulated – they are synthetized as proenzymes requiring activation, and their action is controlled by a group of tissue inhibitors of metalloproteinases (TIMPs). During angiogenesis the degradation of basement membrane and ECM is essential for migration and aligning of EC as well as liberation of ECM-bound pro-angiogenic factors, including VEGF, bFGF and others. MMP-9 is considered to be the most potent pro-angiogenic enzyme, followed by MMP-2. Although the majority of MMPs are involved in tumorigenesis, angiogenesis and cancer progression, some MMPs actions can have pro-apoptotic and anti-angiogenic effects through hampering vessel maturation, releasing pro-apoptotic factors, degrading growth factors or generating anti-angiogenic factors such as angiostatin or endostatin.

Association between MMP and angiogenesis in bladder cancer was first demonstrated by using specific MMP-2 inhibitor Halofuginone in a UBC animal model, which resulted in inhibition of tumor vascularization, invasiveness, and cell proliferation [30]. Levels of tissue MMP-9, MMP-2 and TIMP-1 correlate with high tumor grade and invasion [8, 31]. Overexpression of tissue MMP-9 and TIMP-2 was found to be associated with higher risk of tumor recurrence and poor prognosis [32, 33]. The amount of MMP-2 and MMP-9 in UBC patients' urine is significantly higher in invasive, high-grade cancer and urine MMP-9 was report-ed as an independent prognostic factor of poor overall survival [33]. The value of other urinary MMPs, such as MMP-7 and MMP-1, in detecting UBC and predicting clinical outcome was demonstrated in a number of studies, but the precise role of MMPs in angiogenesis and tumor biology require further evaluation before they can be employed in UBC diagnosis [34, 35].

Thrombospondin-1

As mentioned before, initiation of angiogenesis depends on the balance between pro- and anti-angiogenic factors in the tumor microenvironment. While the function of pro-angiogenic molecules has been extensively described, the role of anti-angiogenic factors is still only partly explained. Among inhibitors of angiogenesis, the role of thrombospondin-1 (TSP-1) has been examined in urological malignancies. Cancer cells often express undetectable to low levels of TSP- 1, and the loss of its expression is described as an important element of the angiogenic switch. TSP-1 belongs to a family of extracellular glycoproteins that serve as adhesive proteins involved in suppression of EC proliferation, migration and vessel-formation. Secreted from tumor as well as stromal cells, TSP-1 inhibits activity of MMP-9 and NO/cGMP-related pathways, limiting their proangiogenic effects. Some reports imply TSP-1 function is more complex: in several cancer types TSP-1 has the capacity to decrease tumor growth and is associated with the early stage, while in others it promotes cancer cell migration and invasion.

In comparison to normal urothelium, the level of TCP-1 in UBC cell lines is decreased despite a relatively unchanged level of VEGF, suggesting that down-regulation of TSP-1 may be the first step in activating the angiogenic switch [36]. Despite some conflicting findings between studies, most authors demonstrated that low TSP-1 expression in UBC is associated with high MVD in tumors, high-grade, tumor invasion, lymph node involvement, disease recurrence and low overall survival [8, 17, 37]. These reports strongly support the concept that TSP-1 is a dominant anti-angiogenic factor involved in suppression of angiogenesis.

Cancer stem cells

Tumors originate from a single cell, but as they grow they become increasingly heterogenic as a result of genetic and phenotypic alterations. There is increasing evidence that among tumor cells exist a small subpopulation of cells with unique features such as the capacity for self-renewal and tumorigenesis, which are crucial for maintenance and growth of tumors. These cells, called cancer stem cells (CSC), have several common properties, including a low prolifera-

tion rate and a high activity of drug efflux pumps that may explain their resistance to classic chemotherapy. CSCs are believed to arise from normal tissue cells or stem cells that underwent malignant transformation, or from dedifferentiated cancers cells that acquired CSC features. Although the exact biological mechanisms are still unclear, emerging evidence suggest that CSCs may support tumor progression by promoting angiogenesis. High CSC content in tumors was found to be responsible for tumor vascular development as a result of increased EC activity and mobilization of bone marrow-derived EPCs. CSCs are also capable of transforming into EPCs, which can undergo further maturation into ECs.

CSCs have been described in bladder cancer but their origin, exact phenotype and role in UBC pathogenesis remain unclear. The first reported phenotype of UBC CSC was CD44+, CK5+, CK20-, but several other markers used for detection of CSC were described, including CD44v6, CD133, 67LR, CD47, CD49, aldehyde dehydrogenaze (ALDH) and cytokeratins 14 and 17 [38, 39, 40]. Although studies analyzing the significance of CSC in UBC pathogenesis revealed unequivocal results, there are reports regarding their prognostic role. It was shown that patents with CK14+ UBC demonstrated reduced overall survival compared to patients with CK14- tumors, and that CK14 overexpression was also associated with recurrence and progression in NMIBC [40]. Patients with a high expression of ALDH1A1 also had increased risk of disease progression and cancer-specific death when compared to patients with low ALDH1A1 [39]. Further validation of UBC CSC markers could lead to their use as predictors of disease progression and

a treatment strategy targeting CSC would be rational. Potential therapeutic options include Heat Shock Protein 90 inhibitors, blocking of COX-PGE2 signaling, blocking of CD47, telomerase inhibitors and the use of retinoids. Development of UBC CSC-oriented therapy is challenging and the majority of studies focusing on this target are still in pre-clinical phases.

CONCLUSIONS

Angiogenesis may be the key to developing novel therapeutic agents able to inhibit molecular pathways responsible for bladder cancer progression. Among these pathways, VEGF seems to play a principal role and ongoing clinical trials with bevacizumab in combination with chemotherapy show the most potential for improving outcome in UBC patients; however, agents targeting other aspects of angiogenesis require further investigation. An understanding of processes underlying angiogenesis will also enable us to better employ pro-angiogenic and anti-angiogenic factors as prognostic markers, allowing us to predict disease progression or recurrence. In this review, we have concentrated on only several molecules and mechanisms involved in tumor angiogenesis, and it must be noted that many other factors regulate formation of tumor vasculature.

References

1. Kurz H, Burri PH, Djonov VG. Angiogenesis and vascular remodeling by intussusception: from form to function. News Physiol Sci. 2003; 18: 65-70.

2. Gao D1, Nolan DJ, Mellick AS, Bambino K, McDonnell K, Mittal V. Endothelial progenitor cells control the angiogenic switch in mouse lung metastasis. Science. 2008; 319: 195-198.

3. Theodoropoulos VE, Lazaris AC, Kastoriotis I, et al. Evaluation of hypoxia-inducible factor 1a overexpression as a predictor of tumour recurrence and progression in superficial urothelial bladder carcinoma. BJU Int. 2005; 95: 425-431.

4. Chai CY, Chen WT, Hung WC, et al. Hypoxia-inducible factor-1alpha expression correlates with focal macrophage infiltration, angiogenesis andunfavourable prognosis in urothelial carcinoma. J Clin Pathol. 2008; 61: 658-664.

5. Deniz H, Karakok M, Yagci F, et al. Evaluation of relationship between HIF-1alpha immunoreactivity and stage, grade, angiogenic profile and proliferative index in bladder urothelial carcinomas. Int Urol Nephrol. 2010; 42: 103-107.

6. Kaelin WG Jr. The von Hippel-Lindau tumor suppressor gene and kidney cancer. Clin Cancer Res. 2004; 10: 6290S-6295.

7. Yang CC, Chu KC, Yeh WM. The expression of vascular endothelial growth factor in transitional cell carcinoma of urinary bladder is correlated with cancer progression. Urol Oncol. 2004; 22: 1-6.

8. Donmez G, Sullu Y, Baris S, et al. Vascular endothelial growth factor (VEGF), matrix metalloproteinase-9 (MMP-9), and thrombospondin-1 (TSP-1) expression in urothelial carcinomas. Pathol Res Pract. 2009; 205: 854-857.

9. Fauconnet S, Bernardini S, Lascombe I, et al. Expression analysis of VEGF-A and VEGF-B: relationship with clinicopathological parameters in bladder cancer. Oncol Rep. 2009; 21: 1495-1504.

10. Kopparapu PK, Boorjian SA, Robinson BD et al. Expression of VEGF and its receptors VEGFR1/VEGFR2 is associated with invasiveness of bladder cancer. Anticancer Res. 2013; 33: 2381-2390.

11. Quentin T, Schlott T, Korabiowska M, et al. 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. 2004; 24: 2745-2756.

12. Sun YW, Xuan Q, Shu Qa, et al. Correlation of tumor relapse and elevated expression of survivin and vascular endothelial growth factor in superficial bladder transitional cell carcinoma. Genet Mol Res. 2013; 12: 1045-1053.

13. Bernardini S, Fauconnet S, Chabannes E, Henry PC, Adessi G, Bittard H. Serum levels of vascular endothelial growth factor as a prognostic factor in bladder cancer. Urol. 2001; 166: 1275-1279.

14. Jeon SH, Lee SJ, Chang SG. Clinical significance of urinary vascular endothelial growth factor in patients with superficial bladder tumors. Oncol Rep. 2001; 8: 1265-1267.

15. Rouanne M, Loriot Y, Lebret T, et al. Novel therapeutic targets in advanced urothelial carcinoma. Crit Rev Oncol Hematol. 2016; 98: 106-115

16. Gan Y, Wientjes MG, Au JL. Expression of basic fibroblast growth factor correlates with resistance to paclitaxel in human patient tumors. Pharm Res. 2006; 23: 1324-1331

17. Shariat SF, Youseff RF, Gupta A, et al. Association of angiogenesis related markers with bladder cancer outcomes and other molecular markers. J Urol. 2010; 183: 1744-1750.

18. Inoue K, Slaton JW, Karashima T. The prognostic value of angiogenesis factor expression for predicting recurrence and metastasis of bladder cancer after neoadjuvant chemotherapy and radical cystectomy. Clin Cancer Res. 2000; 6: 4866-4873

19. Szarvas T, Jäger T, Laszlo V, et al. Circulating angiostatin, bFGF, and Tie2/TEK levels and their prognostic impact in bladder cancer. Urology. 2012; 80: 737.

20. Gazzaniga P, Gandini O, Gradilone, et al. Detection of basic fibroblast growth factor mRNA in urinary bladder cancer: correlation with local relapses. Int J Oncol. 1999; 14: 1123-1127.

21. Billerey C, Chopin D, Aubriot-Lorton MH, et al. Frequent FGFR3 mutations in papillary non-invasive bladder (pTa) tumors. Am J Pathol. 2001; 158: 1955-1959.

22. Bertz S, Abeé C, Schwarz-Furlan S, et al. Increased angiogenesis and FGFR protein expression indicate a favourable prognosis in bladder cancer. Virchows Arch. 2014; 465: 687-695.

23. van Oers JM, Zwarthoff EC, Rehman I, et al. FGFR3 mutations indicate better survival in invasive upper urinary tract and bladder tumours. Eur Urol. 2009; 55: 650-657.

24. Burger M, van der Aa MN, van Oers JM, et al. Prediction of progression of non-muscle-invasive bladder cancer by WHO 1973 and 2004 grading and by FGFR3 mutation status. Eur Urol. 2008; 54: 835-843.

25. Zuiverloon TC, van der Aa MN, van der Kwast TH, et al. Fibroblast growth factor receptor 3 mutation analysis on voided urine for surveillance of patients with low-grade non-muscle-invasive bladder cancer. Clin Cancer Res. 2010; 16: 3011-3018.

26. Inoue K, Slaton JW, Kim SJ, et al. Interleukin 8 expression regulates tumorigenicity and metastasis in human bladder cancer. Cancer Res. 2000; 60: 2290-2299.

27. Zarogoulidis P, Katsikogianni F, Tsiouda T, et al. Interleukin-8 and interleukin-17 for cancer. Cancer Invest. 2014; 32: 197-205.

28. Huang S, Pettaway CA, Uehara H, et al. Blockade of NF-kappaB activity in human prostate cancer cells is associated with suppression of angiogenesis, invasion, and metastasis. Oncogene. 2001; 20: 4188-4197.

29. Karashima T, Sweeney P, Kamat A, et al. Nuclear factor-kappaB mediates angiogenesis and metastasis of human bladder cancer through the regulation ofinterleukin-8. Clin Cancer Res. 2003; 9: 2786-2797

30. Elkin M, Ariel I, Miao HQ, et al. Inhibition of bladder carcinoma angiogenesis, stromal support, and tumor growth by halofuginone. Cancer Res. 1999; 59: 4111-4118

31. Kanayama H, Yokota K, Kurokawa Y, et al. Prognostic values of matrix metalloprote-inase-2 and tissue inhibitor of metallo-proteinase-2 expression in bladdercancer. Cancer. 1998; 82: 1359-1366.

32. Hara I, Miyake H, Hara S, Arakawa S, Kamidono S. Significance of matrix metalloproteinases and tissue inhibitors of metalloproteinase expression in the recurrence of superficial transitional cell carcinoma of the bladder. J Urol. 2001; 165: 1769-1772.

33. Offersen BV, Knap MM, Horsman MR, et al. Matrix metalloproteinase-9 measured in urine from bladder cancer patients is an independent prognostic marker of poor survival. Acta Oncol. 2010; 49: 1283-1287.

34. Szarvas T, Singer BB, Becker M, et al. Urinary matrix metalloproteinase-7 level is associated with the presence of metastasis in bladder cancer. BJU Int. 2011; 107: 1069-1073.

35. Durkan GC, Nutt JE, Rajjayabun PH, et al. Prognostic significance of matrix metalloproteinase-1 and tissue inhibitor of metalloproteinase-1 in voided urinesamples from patients with transitional cell carcinoma of the bladder. Clin Cancer Res. 2001; 7: 3450-3456.

36. Campbell SC, Volpert OV, Ivanovich M, Bouck NP. Molecular mediators of angiogenesis in bladder cancer. Cancer Res. 1998; 58: 1298-1304.

37. Ioachim E, Michael MC, Salmas M, et al. Thrombospondin-1 expression in urothelial carcinoma: prognostic significance and association with p53alterations, tumour angiogenesis and extracellular matrix components. BMC Cancer. 2006; 6: 140.

38. Yang YM, Chang JW. Bladder cancer initiating cells (BCICs) are among EMA-CD44v6+ subset: novel methods for isolating undetermined cancer stem (initiating) cells. Cancer Invest. 2008; 26: 725-733.

39. Su Y, Qiu Q, Zhang X, et al. Aldehyde dehydrogenase 1 A1-positive cell population is enriched in tumor-initiating cells and associated with progression of bladder cancer. Cancer Epidemiol Biomarkers Prev. 2010; 19: 327-337.

40. Volkmer JP, Sahoo D, Chin RK, et al. Three differentiation states risk-stratify bladder cancer into distinct subtypes. Proc Natl Acad Sci USA. 2012; 109: 2078-2083. ■