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Angiogenesis, Lymphangiogenesis and Lymphovascular Invasion: Prognostic Impact for Bladder Cancer Patients

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1. Introduction

Bladder cancer is the second most common tumor of the urogenital tract. Urothelial carcinoma is the most frequent histologic type, being unique among epithelial carcinomas in its divergent pathways of tumorigenesis. Surgery continues to have a predominant role in the management of urothelial bladder cancer (Kaufman et al., 2009). However, the debate about the best treatment approach for T1G3 and muscle invasive tumors continually challenges all urologic surgeons and oncologists. This debate involves several aspects. First, a significant number of T1G3 tumors recurs and progresses rapidly after transurethral resection and BCG treatment (Wiesner et al., 2005). Second, half of patients with invasive tumors have a dismal outcome despite an effective treatment by radical cystectomy (Sternberg et al., 2007). Third, the extension of lymphadenectomy remains an issue of controversy, although clinical evidence suggests that an extended lymph node dissection may not only provide prognostic information, but also a significant therapeutic benefit for both lymph node-positive and lymph node-negative patients undergoing radical cystectomy (May et al., 2011). In muscle invasive bladder cancer, the presence of tumor foci in lymph nodes is an early event in progression, and the lymphatic vessels within or in the proximity to the primary tumor serve as the primary conduits for tumor dissemination (Youssef et al., 2011). Fourth, although urothelial bladder cancer is a chemo-sensitive tumor (Kaufman et al., 2000; von der Maase et al., 2000), adjuvant systemic chemotherapy does not reveal benefits (Walz et al., 2008), and neoadjuvant chemotherapy is not yet accepted as the best approach in invasive bladder cancer (Clark, 2009). Therefore, in order to solve the aforementioned problems, it is crucial to improve the knowledge about tumor microenvironment, regulation of cancer metabolism and neovascularization.

Blood and lymphatic neovascularization are essential for tumor progression and metastasis, by promoting oxygenation and fluid drainage, and establishing potential routes of dissemination (Adams and Alitalo, 2007). Therefore, the inhibition of tumor-induced neovascularization represents a powerful option for target therapy, in order to restrain the most efficient pathway of cancer spread.

2. Angiogenesis and lymphangiogenesis: Molecular regulation of vasculature development

During embryogenesis, the formation of the blood vascular system initiates by vasculogenesis: haemangioblasts proliferate, migrate and differentiate into endothelial cells, which in turn will organize a primitive vascular plexus. In parallel, angiogenesis promotes the remodeling and expansion of the primary capillary network, originating a hierarchical structure of different sized vessels that will mature into functional capillaries, veins and arteries (Risau, 1997). The lymphatic vascular system develops latter, when a group of blood endothelial cells differentiates into a lymphatic endothelium that subsequently sprouts to form the primary lymph sacs. By lymphangiogenesis, the lymphatic endothelial cells from the lymph sacs will further sprout, originating the peripheral lymphatic system (Sabin, 1902, as cited by Oliver & Detmar, 2002).

During postnatal life, blood and lymphatic vascular systems are, normally, in a quiescent state. Physiological angiogenesis and/or lymphangiogenesis occur to maintain or restore the integrity of tissues, namely during wound healing and the ovarian cycle. Conversely, the neovascularization machinery may be activated in pathological processes such as cancer and inflammatory diseases (reviewed in Lohela et al., 2009).

Similarly to physiological neovascularization, tumor-induced angiogenesis and/or lymphangiogenesis occur to satisfy the metabolic demands of a new tissue — the malignant tissue. Therefore, the molecular factors involved in the formation of the vascular systems during embryogenesis are newly recruited by the growing tumor (Papetti & Herman, 2002).

2.1 From angiogenesis to lymphangiogenesis in the embryo

The proliferation, sprouting and migration of endothelial cells during vasculogenesis and angiogenesis is mainly guided by the vascular endothelial growth factor (VEGF) signaling through VEGF receptor-2 (VEGFR-2) (Risau, 1997).

VEGF (or VEGF-A), initially termed as vascular permeability factor (VPF) (Senger et al., 1983), is a specific mitogen and pro-survival factor for blood endothelial cells, also stimulating vascular permeability. It binds and activates two tyrosine kinase receptors primarily found on the blood endothelium: VEGFR-1 (or Flt-1, fms-like tyrosine kinase 1) and VEGFR-2 (or KDR/Flk-1, human kinase insert domain receptor/mouse foetal liver kinase 1) (reviewed in Carmeliet, 2005). Interaction of VEGF with VEGFR-1 negatively regulates vasculogenesis and angiogenesis during early embryogenesis (Fong et al., 1999). On the contrary, VEGFR-2 is the earliest marker for endothelial cell development: mouse embryos lacking VEGFR-2 die at embryonic day 8.5-9.5 due to no development of blood vessels as well as very low hematopoiesis (Shalaby et al., 1995). Regarding the ligand, even heterozygote mice for *Vegf* deficiency die at embryonic day 11-12: blood islands, endothelial cells and vessel-like tubes fail to develop (Carmeliet et al., 1996; Ferrara et al., 1996).

In humans, five weeks after fertilization, certain blood endothelial cells become responsive to lymphatic inducing-signals. The lymphatic vessel endothelial hyaluronan receptor-1 (LYVE-1), a CD44 homologous transmembrane protein, is the first marker of lymphatic endothelial commitment. Initially, it is evenly expressed by the blood endothelium of the cardinal vein, which causes the blood endothelium to acquire the ability to differentiate in lymphatic endothelium (Banerji et al., 1999). The polarized expression of the prospero related homeobox gene-1 (Prox-1) transcription factor in a subpopulation of blood endothelial cells determines the establishment of the lymphatic identity and initiates the formation of the lymphatic vascular system. In mice, Prox-1 expressing cells are first observed at embryonic day 10 in the jugular vein (Wigle & Oliver, 1999). *Prox1* deletion leads to a complete absence of the lymphatic vasculature (Wigle et al., 2002). The expression of the transcription factor Sox18 [SRY (sex determining region Y) box 18] acts as a molecular switch to induce differentiation of lymphatic endothelial cells: it activates Prox-1 transcription by binding to its proximal promoter. Sox18-null embryos show a complete blockade of lymphatic endothelial cell differentiation (François et al., 2008). Later, the sprouting, migration and survival of the newly formed lymphatic endothelial cells depends on the expression of VEGF-C by the mesenchymal cells surrounding the cardinal veins (Karkkainen et al., 2004) (Fig. 1).

VEGF-C, like VEGF, is a member of the VEGF family of growth factors and a mitogen for lymphatic endothelial cells. VEGF-D is also a pro-lymphangiogenic factor, although its deletion does not affect the development of the primitive lymphatic vessels (Baldwin et al. 2001). Conversely, in *Vegfc-/-* mice, Prox-1 positive cells appear in the cardinal veins, but fail to migrate and proliferate to form primary lymph sacs (Karkkainen et al., 2004). VEGF-C and VEGF-D interact with VEGFR-3 (of Flt-4, fms-like tyrosine kinase 4). Their affinity to VEGFR-3 is increased by proteolytic cleavage; the fully processed forms can also bind to VEGFR-2 (reviewed in Lohela et al., 2009).

VEGFR-3 is widely expressed at the early stages of embryonic blood vasculature, becoming virtually restricted to lymphatic endothelium in the later stages of embryonic development, (after the lymphatic commitment mediated by Prox-1 expression), and during adult life (Kaipainen et al., 1995). In mice, inhibition of VEGFR-3 expression at embryonic day 15 induces regression of the developing lymphatic vasculature by apoptosis of lymphatic endothelial cells (Makinen et al., 2001).

The subsequent development of the lymphatic vasculature involves the separation of the blood and lymphatic vascular systems, the maturation of lymphatic vessels and the formation of secondary lymphoid organs. The molecular regulation of these processes involves the coordinated expression of distinct genes from those involved in the early events of lymphangiogenesis (reviewed in Alitalo et al., 2005) (Fig. 1). Moreover, several other growth factors, namely cyclooxygenase-2 (COX-2) fibroblast growth factor-2 (FGF-2), hepatocyte growth factor (HGF), insulin-like growth factors (IGFs) and platelet-derived growth factor-B (PDGF-B) have been shown to induce lymphangiogenesis and/or angiogenesis in experimental models (reviewed in Cao, 2005). These are mainly protein tyrosine kinases, which play central roles in signal transduction networks and regulation of cell behavior. In the lymphatic endothelium, these tyrosine kinases are collectively involved in processes such as the maintenance of existing lymphatic vessels, growth and maturation of new vessels and modulation of their identity and function (Williams et al., 2010).
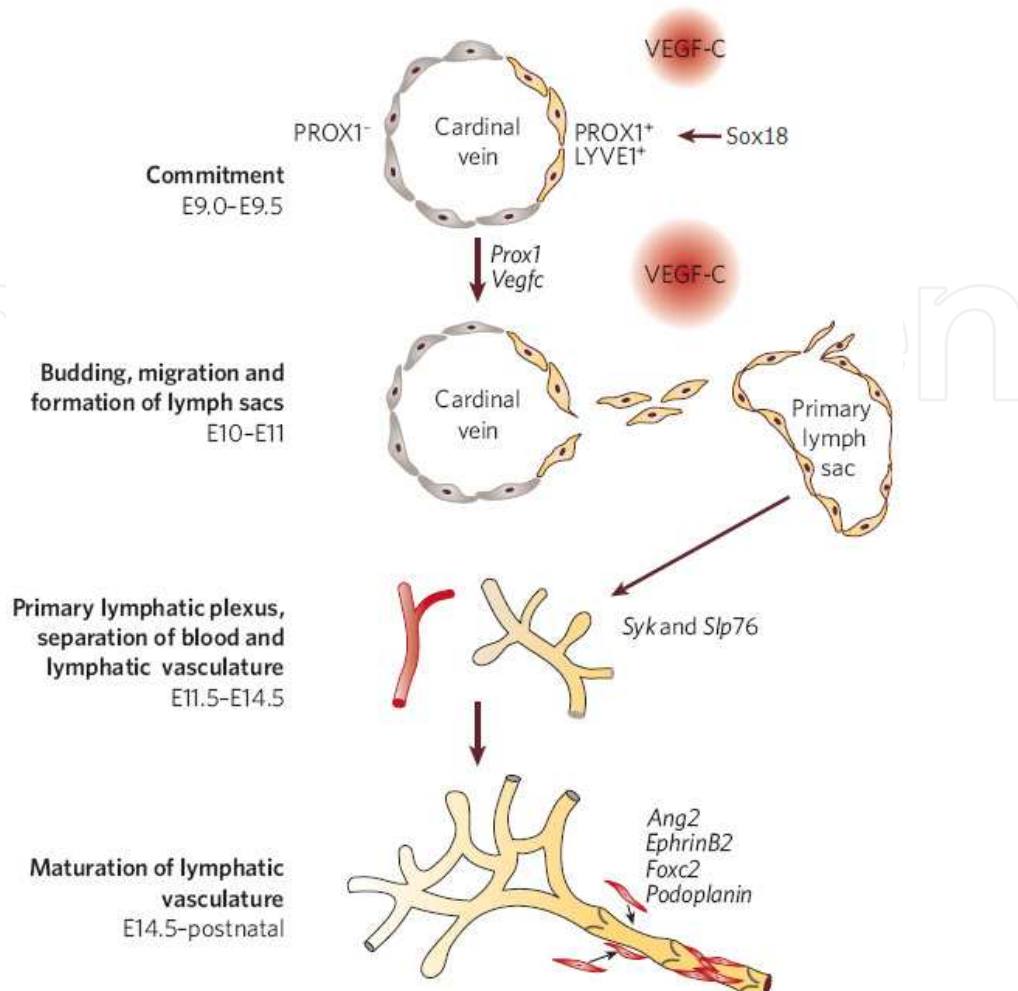
Fig. 1. Model for the development of mouse lymphatic vasculature (E- embryonic day; Syk-protein-tyrosine kinase SYK; Slp76- SH2 domain-containing leucocyte protein, 76-kDa; Ang2- angiopoietin 2; Foxc2- Forkhead Box C2) (adapted by permission from © 2005 Nature Publishing Group. Originally published in *Nature*. 438: 946-953)

2.2 Promotion of angiogenesis and lymphangiogenesis in the malignancy context

The major cause of cancer mortality is the metastatic spread of tumor cells that can occur via multiple routes, including blood and lymphatic vasculatures. For metastasis to occur, selected clones of malignant cells must be able to invade the newly formed vessels and disseminate. Induction of angiogenesis and/or lymphangiogenesis is, therefore, one of the first steps of the metastatic cascade (Alitalo & Carmeliet, 2002; Tobler & Detmar, 2006).

During the pre-vascular phase, the malignant tumor remains small (up to 1 or 2 mm^3); the preexistent surrounding blood vessels ensure the supply of oxygen and nutrients necessary for its survival. However, the expansion of the tumor mass is angiogenesis-dependent. As a compensatory response to hypoxia, proangiogenic factors such as VEGF are released by the malignant cells and infiltrating immune cells, namely monocytes. As a result, angiogenesis occurs and the tumor acquires its own blood supply. Neoplastic growth is thus promoted, as well as the potential for invasion and haematogenic metastasis (Kerbel, 2000).

Vegf is upregulated in hypoxia via the oxygen sensor hypoxia-inducible factor (HIF)-1α (Pugh & Ratcliffe, 2003). Another recently described VEGF activation mechanism is the induction of

the transcriptional coactivator peroxisoma proliferator-activated receptor-gamma coactivator-1α (PGC-1α) in response to the lack of nutrients and oxygen (Arany et al., 2008). Additionally, VEGF gene expression can be upregulated by oncogene signaling, several growth factors, inflammatory cytokines and hormones (reviewed in Ferrara, 2004). Tumor cells secrete VEGF mainly in a paracrine manner, although it can also act in an autocrine manner to promote a protective/survival effect to endothelial cells, among other cell types (Brusselmans et al., 2005). The mechanisms underlying tumor lymphangiogenesis are not clearly defined. Inflammation seems to promote lymphatic neovascularization: inflammatory cells that infiltrate in the growing tumor produce lymphangiogenic growth factors. Another lymphangiogenesis trigger mechanism may be the high interstitial pressure generated inside the tumors due to the excessive production of interstitial fluid (reviewed in Cao, 2005). On the other hand, the extracellular matrix is of central importance for the generation of new lymphatic vessels as a response to the pathological stimulus. Integrins, a superfamily of cell adhesion molecules, are able to influence cell migration: integrin α9β1 is a target gene for Prox1, and its direct binding to VEGF-C and VEGF-D stimulates cell migration (reviewed in Wiig, 2010).

VEGF-C and VEGF-D, via signaling through VEGFR-3, appear to be essential for tumor-associated lymphangiogenesis, leading to lymphatic vessel invasion, lymph node involvement and distant metastasis (reviewed in Achen & Stacker, 2008). Moreover, VEGF interaction with VEGFR-2 may also promote lymphatic neovascularization, namely inside the regional draining lymph nodes, even before lymph node metastasis occurrence. This probably corresponds to a pathophysiologic strategy of "soil" preparation by the primary tumor to ensure the success of its future dissemination (Hirakawa et al., 2005). In fact, sentinel lymph node metastasis is the first step in the spreading of many cancer types.

Preexisting blood and lymphatic vessels in the vicinity of the malignant mass may contribute to tumor spread. However, *de novo* formed vessels by tumor-induced angiogenesis and lymphangiogenesis seem to be the preferential routes for dissemination (reviewed in Cao, 2005). This is a consequence of the ultra-structure of the tumor-associated blood and lymphatic vessels.

2.3 Ultra-structure of tumor-associated blood and lymphatic vessels

Blood vessels present in malignant tissues show remarkable differences with vessels present in normal tissues. Tumor blood vessels are highly disorganized: they are tortuous, excessively branched and dilated. The basement membrane and the muscular coverage are incomplete or absent. The endothelial cells, abnormal in shape, overlap and are projected into the lumen rather than organizing a pavement layer below the basement membrane. Blood vessel invasion is facilitated by this aberrant structure, but the extravasation rate is high, and blood flow is variable. As a result, interstitial tumor hypertension occurs, and delivery of therapeutic agents into tumors is compromised (Jain & Carmeliet, 2001; reviewed in Cao, 2005). The intratumoral edema is pernicious to malignant cells; therefore, homeostasis needs to be re-established. The formation of a tumoral lymphatic vasculature could potentially resolve this problem.

The key function of lymphatic vessels is to collect the excessive amount of interstitial fluid back to the blood circulation for immune surveillance in lymph nodes. Unlike normal blood capillaries, lymphatic capillaries have a discontinuous or fenestrated basement membrane and are not ensheathed by pericytes or smooth muscle cells; the endothelial cells are arranged in a slightly overlapping pattern and lack tight interendothelial junctions. Specialized anchoring

filaments of elastic fibers connect the endothelial cells to the extracellular matrix, which causes the vessels to dilate rather than to collapse when hydrostatic pressure rises (Alitalo et al., 2005; Tobler & Detmar, 2006). This structure facilitates the collection of interstitial fluid and is ideal for malignant cells' entry into the lymphatic flow.

A highly debated question is whether there are functional lymphatic vessels inside tumors (reviewed in Alitalo & Carmeliet, 2002; reviewed in Detmar & Hirakawa, 2002). On one hand, the elevated interstitial pressure generated by the proliferation of the malignant cells and by the high extravasion rate compromises the infiltration of new lymphatic vessels in the tumor stroma. Although intratumoral lymphangiogenesis may occur, the newly formed vessels are compressed and nonfunctional (Jain & Fenton, 2002). To compensate the lack of an intratumoral draining mechanism, the peritumoral lymphatic vessels enlarge due to an excess of pro-lymphangiogenic factors in that area. Therefore, in this model, the peritumoral lymphatic vessels passively collect interstitial fluid and, eventually, malignant cells (Carmeliet & Jain, 2000) (Fig. 2, A). However, some studies have demonstrated a relationship between the existence of functional intratumoral lymphatics, with cycling lymphatic endothelial cells and tumor emboli, and lymph node involvement (reviewed in Da et al., 2008). Additionally, peritumoral lymphangiogenesis occurs, and the new vessels actively contribute to metastatic spread (Padera et al., 2002) (Fig. 2, B). Probably, there are some organ-specific determinants that influence the occurrence of peritumoral and/or intratumoral lymphangiogenesis, as well as the function of the newly formed vessels.

2.4 Lymphovascular invasion and metastasis

Tumor metastasis involves a coordinated series of complex events that include promotion of angiogenesis and lymphangiogenesis, detachment of malignant cells from the primary tumor, microinvasion of the surrounding stroma, blood and/or lymphatic vessel invasion, survival of the malignant cells in the blood and/or lymphatic flow, and extravasion and growth in secondary sites. Because the large lymphatic vessels reenter the blood vascular system, malignant cells spread via the lymphatic system to the regional lymph nodes and, from this point, to distant organs (Alitalo & Carmeliet, 2002; Tobler & Detmar, 2006) (Fig. 3). Follow-up data have shown that 80% of the tumors, mainly those of epithelial origin, disseminate through the lymphatic vasculature; the remaining 20% use the blood circulation to colonize secondary organs (reviewed in Saharinen et al., 2004; reviewed in Wilting et al., 2005).

The blood vessels are not the best route for the success of malignant dissemination. Although their disorganized structure may contribute to the intravasion of malignant cells or emboli, in the bloodstream these cells experience serum toxicity, high shear stresses and mechanical deformation. Consequently, the viability of the tumor cells is seriously compromised (reviewed in Swartz, 2001). Conversely, the success rate of lymphogenous spread is high. As previously referred, the structure and function of the lymphatic capillaries facilitates intravasion of tumor cells or emboli. On the other hand, the composition of the lymph is similar to interstitial fluid, which provides an optimal medium for the survival of malignant cells. In collecting lymphatic vessels, muscle fibers assure lymph propulsion, that flows slowly, and valves prevent its backflow. Lymph nodes are areas of flow stagnation that represent ideal "incubators" for malignant cells' growth. Some cells exit the lymph node through the efferent channels or high endothelial venules. Other cells may remain mechanically entrapped for long periods of time, originating
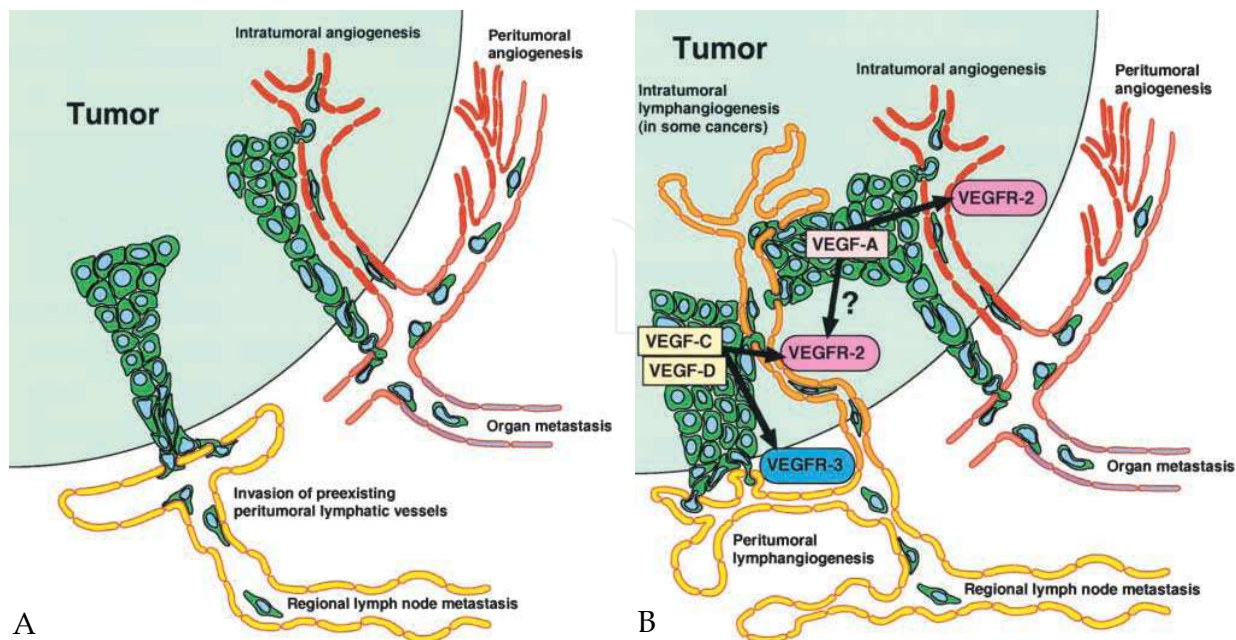
Fig. 2. (A) Traditional model of tumor metastasis via lymphatic and blood vessels. (B) Active lymphangiogenesis model of tumor metastasis (reprinted by permission from © 2002 Rockefeller University Press. Originally published in *J. Exp. Med.* 196: 713-718)
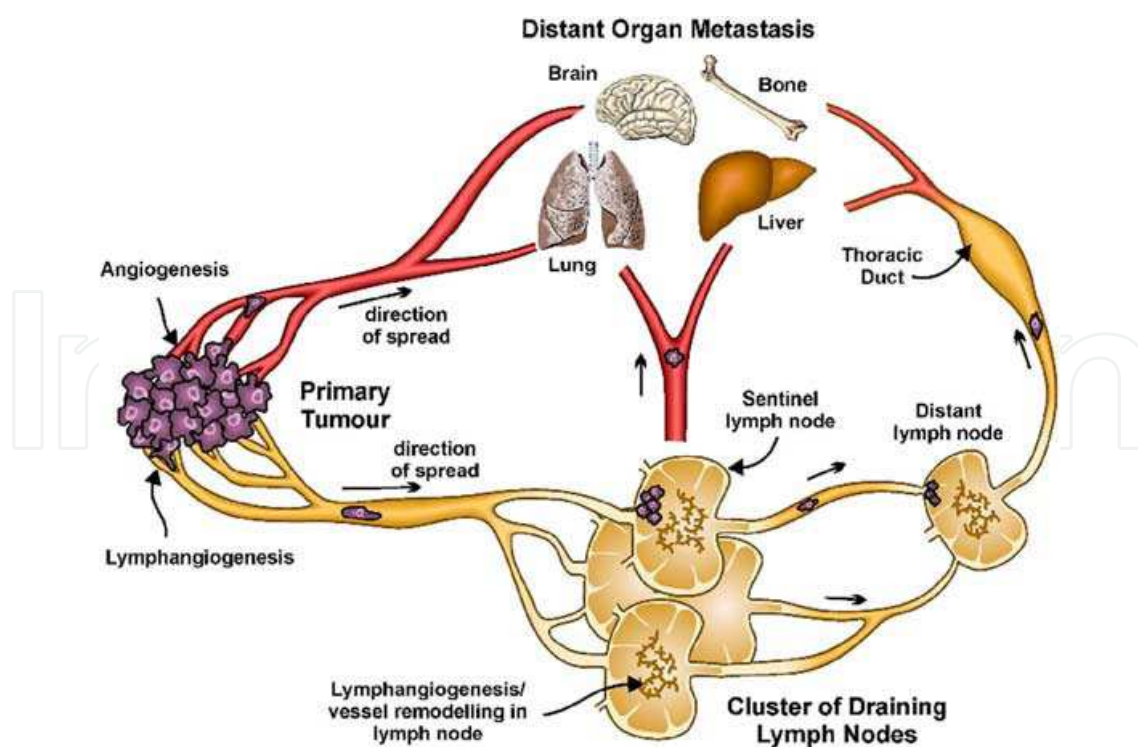

Fig. 3. Pathways of dissemination of malignant cells (reprinted by permission from © 2008 John Wiley & Sons, Inc. Originally published in *Ann. N. Y. Acad. Sci.* 1131: 225-234)

micrometastases (Swartz, 2001; Van Trapen & Pepper, 2002). Martens and colleagues described the expression of a gene signature of scavenger and lectin-like receptors in the lymph node sinus, which are known mediators of tumour cell adhesion and, therefore, can contribute to selective metastasis in an organ-specific context (Martens et al., 2006). Probably, tumor-cell-specific characteristics, microenvironmental factors and crosstalk between tumor and host cells have a pivotal role in determining survival and growth of micrometastasis. Moreover, lymph node lymphangiogenesis may provide an additional mechanism to facilitate further metastatic spread throughout the lymphatic system (Ji, 2009). The occurrence of lymphangiogenesis prior to arrival of tumor cells indicates that signals derived from the primary tumor are transported to the draining lymph nodes (Hirakawa et al., 2005).

Different tumors metastasize preferentially to different organs, suggesting that tumor spread is a guided process. It has been reported that malignant cells may use chemokine receptor ligand interactions to guide the colonization of target organs (reviewed in Saharinen et al., 2004; reviewed in Achen & Stacker, 2008). Chemokines are a family of chemoattractant cytokines that bind to G protein-coupled receptors expressed on target cells, namely malignant cells (Laurence, 2006). For instance, breast cancer cells, that normally choose regional lymph nodes, bone marrow, lung and liver as their first sites of destination, overexpress CCR7 (chemokine, CC motif, receptor 7) and CXCR4 (chemokine, CXC motif, receptor 4). Their ligands, SLC/CCL2 (secondary lymphoid chemokine / CC-type chemokine ligand 21) and SDF-1 CXCL12/ (stromal cell-derived factor 1 / chemokine, CXC motif, ligand 12) are expressed at high levels by isolated lymphatic endothelial cells and lymphatic endothelium from vessels present in the preferred sites of metastasis (Muller et al., 2001). This guides chemoattraction and migration of tumor cells, and characterizes lymphatic vessel invasion as an active event.

3. Angiogenesis, lymphangiogenesis and lymphovascular invasion in urothelial bladder cancer

The metastatic profile of urothelial bladder carcinoma implies, as in most malignant tumors, the dissemination of tumor cells through the lymphatic vasculature, and the colonization of regional lymph nodes is an early event in progression. Smith & Whitmore reported the involvement of the internal iliac and obturator groups of lymph nodes in about 74% of patients who underwent radical cystectomy; the external iliac nodes were involved in 65% of the patients, and the common iliac nodes were involved in 20% of the cases (Smith & Whitmore, 1981). As already referred, controversy exists regarding the optimal extent of lymphadenectomy and the number of lymph nodes to be retrieved at radical cystectomy. An extended pelvic lymph node dissection (encompassing the external iliac vessels, the obturator fossa, the lateral and medial aspects of the internal iliac vessels, and at least the distal half of the common iliac vessels together with its bifurcation) has been suggested as potentially curative in patients with metastasis or micrometastasis to a few nodes (Karl et al., 2009; Abol-Enein et al., 2011). Wright and colleagues observed that an increased number of lymph nodes removed at the time of radical cystectomy associates with improved survival in patients with lymph node-positive bladder cancer (Wright et al., 2008). The recommendation from the Bladder Cancer Collaboration Group is that ten to fourteen lymph nodes should be removed at the time of radical cystectomy (Herr et al., 2004). The concept of lymph node density (the number of positive lymph nodes divided by the total number of lymph nodes) was introduced by Stein and colleagues and helps to select lymph node-positive patients after radical

cystectomy for adjuvant treatment (Stein et al., 2003). However, the lymph node density threshold is a debatable question (Gilbert, 2008). In large series, the median number of total lymph nodes removed was nine, with high lymph node density (25%), which can lead to misleading N0 staging (Wright et al., 2008). Therefore, in this subgroup of patients (lymph nodes removed ≤ 9 and N0), another prognostic factor is needed to better select patients for adjuvant treatment. Moreover, according to Malmström, extending the boundaries of surgery will not drastically improve survival. The focus should be on exploring biomarkers that predict extravesical dissemination and improving on the systemic treatment concept (Malmström, 2011). In this line of investigation, angiogenesis, lymphangiogenesis and lymphovascular invasion occurrence have been implicated in bladder cancer progression, invasion and metastasis, and represent potential targets for guided therapy.

Several studies reported a significant association between VEGF overexpression — both in tumor tissue (Crew et al., 1997; O'Brien et al., 1995) and urine (Crew et al., 1999; Jeon et al., 2001) —, high blood vessel density (Goddard et al., 2003; Santos et al., 2003) and the occurrence of recurrence and progression in patients with non-muscle invasive bladder cancer. In this group of patients, it has been observed that angiotensin II type 1 receptor (AT1R) expression associates with high blood vessel density and is related to early intravesical recurrence (Shirotake et al., 2011). AT1R supports tumor-associated macrophage infiltration, which results in enhanced tissue VEGF protein levels (Egami et al., 2009). These results suggest that AT1R is involved in bladder tumor angiogenesis and may become a new molecular target and a prognostic factor for urothelial bladder cancer patients

In the subset of invasive urothelial bladder cancer, most studies also reported the association between angiogenesis occurrence and unfavorable prognosis. High blood vessel density was identified as an independent prognostic factor by several authors (Bochner et al., 1995; Chaudhary et al., 1999; Dickinson et al., 1994; Jaeger et al., 1995). Moreover, overexpression of VEGF associates with high blood vessel density (Sato et al., 1998; Yang et al., 2004). Analysis of serum levels of VEGF has demonstrated its optimal sensitivity and specificity for predicting metastatic disease (Bernardini et al., 2001). Inoue and colleagues reported the importance of measuring blood vessel density and VEGF immunoexpression in identifying patients with invasive tumors who are at high risk of recurrence and development of metastasis after radical cystectomy and neoadjuvant systemic chemotherapy. The author highlighted the role of VEGF as a cell survival factor, not only by protecting the malignant cells in situations of hypoxia, but also during the occurrence of chemotherapy-induced apoptosis (Inoue et al., 2000).

Beyond VEGF signaling, other angiogenesis-related molecules have been implicated in bladder cancer recurrence, progression and metastasis, namely several proangiogenic factors — matrix metalloproteinases, fibroblast growth factors, platelet derived-growth factors, cyclooxygenases, integrins, angiopoietins, Notch signaling — and several antiangiogenic factors — thrombospondin-1, angiostatin-endostatin, platelet factor-4 (Chikazawa et al., 2008; Durkan et al., 2001; Grossfeld et al., 1997; Patel et al., 2006; reviewed in Pinto et al., 2010; Shariat et al., 2010).

The relevance of lymphangiogenesis in bladder cancer setting has gained recent attention. A few articles suggest that lymphangiogenesis occurrence, detected using specific lymphatic markers, is associated with poor prognosis (Fernández et al., 2008; Ma et al., 2010; Miyata et al., 2006; Zhou et al., 2011; Zu et al., 2006). VEGF-C, VEGF-D and VEGFR-3 are overexpressed in bladder cancer and promote tumor-induced lymphangiogenesis. This correlates with tumor upstaging and lymph node involvement, and results in a worse

prognosis (Afonso et al., 2009; Miyata et al., 2006; Suzuki et al., 2005; Herrmann et al., 2007; Zhou et al., 2011; Zu et al., 2006). Interestingly, VEGF-C overexpression also associates with angiogenic events, probably by interaction of the fully processed form with VEGFR-2 (Afonso et al., 2009; Miyata et al., 2006). On the other hand, tumor associated macrophages play an important role in promoting lymphangiogenesis by producing VEGF-C and VEGF-D, mainly in peritumoral areas (Schoppmann et al., 2002). The blockade of VEGF-C/D with a soluble VEGF receptor-3 markedly inhibited lymphangiogenesis and lymphatic metastasis in an orthotopic urinary bladder cancer model. In addition, the depletion of tumor associated macrophages exerted similar effects (Yang et al. 2011).

Lymphovascular invasion has been identified as an independent prognostic factor for bladder cancer patients in several studies (Cho et al., 2009; Leissner et al., 2003; Lotan et al., 2005; Quek et al., 2005). In patients with newly diagnosed T1 urothelial bladder cancer, lymphovascular invasion in transurethral resection of bladder tumor specimens predicts disease progression and metastasis (Cho et al., 2009). Lotan and colleagues observed that blood and lymphatic vessel invasion (accessed by Haematoxylin-eosin stain) is an independent predictor of recurrence and low overall survival in patients who undergo radical cystectomy for invasive urothelial bladder cancer and are lymph node negative. They emphasized that these patients represent a high risk group that may benefit from neoadjuvant or adjuvant treatments. However, in this study, the mean number of lymph nodes removed per patient at the time of radical cystectomy was 20,1±10,2 (Lotan et al., 2005).

The prognostic impact of lymphovascular invasion in patients with lymph node-negative urothelial bladder cancer treated by radical cystectomy has been recently validated in large multicentre trials (Bolenz et al., 2010; Shariat et al, 2010). May and colleagues emphasized that, besides the importance of performing extended lymphadenectomies, the information resulting from an assessment of lymphovascular invasion is critical for stratification of risk groups and identification of patients who might benefit from adjuvant treatments (May, 2011). Algaba underlined that, in this field, it would be necessary to reach a consensus on strict diagnostic criteria as soon as possible, to be able to incorporate this prognostic factor in clinical practice (Algaba, 2006). Leissner and colleagues endorsed that blood and lymphatic vessel invasion should be commented on separately in the pathology report (Leissner et al., 2003).

Afonso and colleagues reported the prognostic contribution of molecular markers of blood vessels (like CD31) (Fig. 4, A) and lymphatic vessels (like D2-40) (Fig. 4, B) to accurately assess the occurrence of blood and/or lymphatic vessel invasion. The use of endothelial markers is encouraged because immunohistochemistry antibodies are significantly more sensitive in detecting invasive events than the standard Haematoxylin-eosin staining method and, additionally, facilitate the discrimination between blood and lymphatic vessel invasion. This is particularly important in identifying isolated malignant cells invading lymphatic vessels, because their viability is more probable in the lymphatic flow than in the blood circulation. Conversely, emboli of malignant cells are better suited to survive in the bloodstream, and are more easily identified, even by the traditional Haematoxylin-eosin staining method. This advocates the use of lymphatic markers for purposes of counting invaded lymphatic vessels. In this study, blood vessel invasion by malignant emboli assessed by CD31 staining (Fig. 5, A), and lymphatic vessel invasion by isolated malignant cells assessed by D2-40 staining (Fig. 5, B) significantly affected patients' prognosis; blood vessel invasion remained as an independent prognostic factor (Afonso et al., 2009). When included in a model of bladder cancer aggressiveness, these parameters contributed to a clear separation between low and high aggressiveness groups (Afonso et al., 2011).
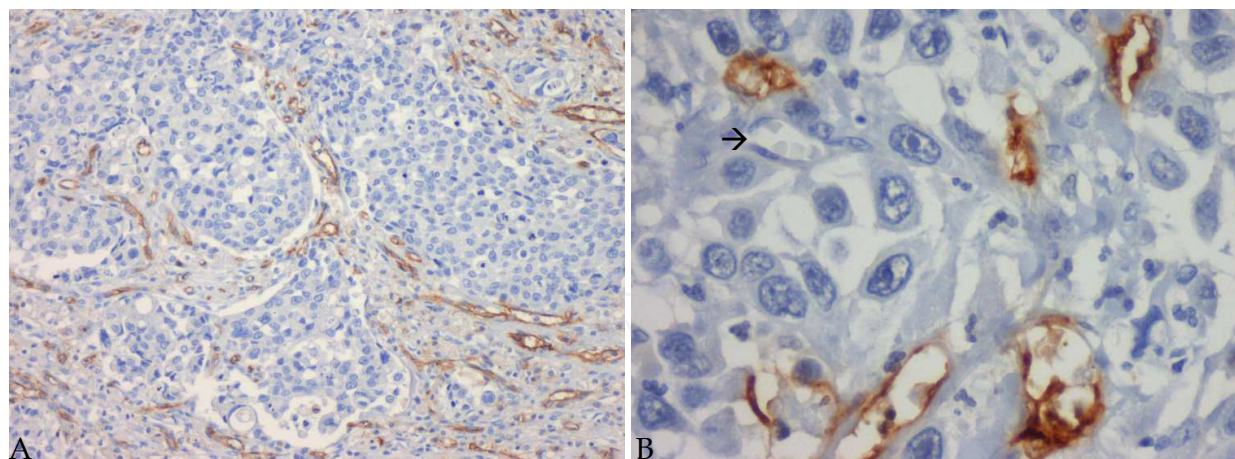
Fig. 4. Intratumoral blood vessels highlighted by CD31 (A), and intratumoral lymphatic vessels highlighted by D2-40 (B), in invasive urothelial bladder carcinoma. Evidence of internal negative control in A (D2-40 negative blood vessel →) (original magnification x100) (reprinted by permission from © 2009 John Wiley & Sons, Inc. Originally published in *Histopathol*. 55: 514-524)

Both peritumoral and intratumoral lymphatic vessels seem to be functional for urothelial cells' dissemination. Some articles reported the existence of intratumoral lymphatic vessels in bladder tumors, and their possible participation in metastatic events. No intratumoral edema has been observed, which is consistent with the occurrence of efficient lymphatic neovascularization (Afonso et al., 2009; Fernández et al., 2008; Ma et al., 2010; Miyata et al. 2006). Lymphatic vessel invasion occurrence correlates with high lymphatic vessel density values, mainly in the intratumoral areas. Although most of the invaded lymphatic vessels were distorted and collapsed, single malignant cells were significantly observed in the well-preserved intratumoral lymphatic vessels (Fig. 5, B). Moreover, the absence of intratumoral edema is a surrogate marker of an efficient lymphatic flow (Afonso et al., 2009).
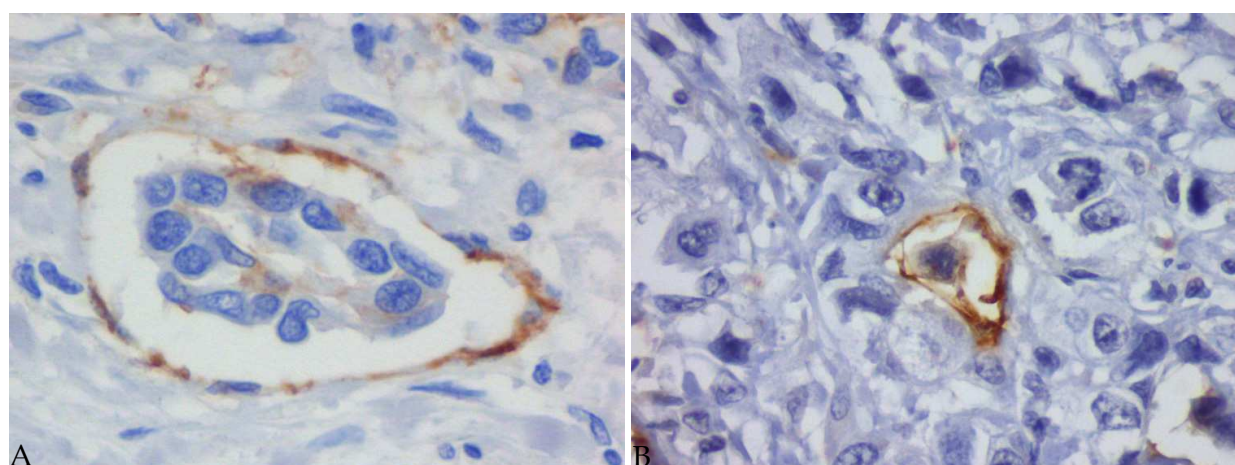

Fig. 5. Intratumoral blood vessel highlighted by CD31 invaded by a small malignant embolus (A), and intratumoral lymphatic vessel highlighted by D2-40 invaded by an isolated malignant cell (B), in invasive urothelial bladder carcinoma (original magnification x100) (reprinted by permission from © 2009 John Wiley & Sons, Inc. Originally published in *Histopathol*. 55: 514-524)

4. Angiogenesis and Lymphangiogenesis as therapeutic targets in urothelial bladder cancer

Our current understanding of the importance of tumor-induced angiogenesis and lymphangiogenesis for the occurrence of haematogenous and lymphogenous metastasis suggests that, by blocking the activity of key molecules involved in these processes, it should be possible to suppress the onset of metastasis following diagnosis of cancer and its subsequent therapy. Moreover, prophylactic suppression of metastasis would be useful for patients who are at risk of recurrence (Thiele & Sleeman, 2006). Therefore, clinical trials evaluating novel agents and combinations including chemotherapeutic drugs, as well as targeted inhibitors, are desperately needed (Iyer et al., 2010).

Two types of neovascularization inhibitors have been described. The direct inhibitors refer to compounds that function directly on endothelial cells by blocking a common pathway of vessel growth. Indirect inhibitors are molecules that neutralize the functions of angiogenic and lymphangiogenic growth factors; due to their mode of action, these are preferred over the direct inhibitors (Cao, 2005; Folkman, 2003). The main strategies that have been tested focus on modulating the signaling of VEGF family of growth factors and receptors, and are based on the use of monoclonal antibodies or soluble versions of receptors to neutralize the ligand-receptor interaction, and the inhibition of the kinase activity of the receptors (Achen et al., 2006; Thiele & Sleeman, 2006).

In 2004, the U.S. Food and Drug Administration (FDA) has approved bevacizumab (Avastin®), a humanized monoclonal antibody that binds to VEGF-A, as the first drug developed solely for antiangiogenesis anticancer use in humans. Antiangiogenic drugs are presently approved in a wide number of tumor types, namely in breast, colorectal, lung, liver, glioblastoma and kidney cancer. Other compounds are currently in preclinical development, with many of them now entering the clinic and/or achieving approval (reviewed in Boere et al., 2010; reviewed in Cook & Figg, 2010; reviewed in Pinto et al., 2010).

In anticancer therapy, an angiogenesis inhibitor may prevent the growth of new blood vessels. This should decrease the delivery of oxygen and nutrients – the "starving therapy" – which are indispensable elements for the support of uncontrolled cell division and tumor expansion. Angiogenesis inhibitors are predicted to be cytostatic, stabilizing tumors and perhaps preventing metastasis, rather than being curative (Zhi-chao & Jie, 2008). Therefore, there is the need to administrate this type of therapy for long periods of time. As a consequence, problems with bleeding, blood clotting, heart function and depletion of the immune system are common (Cohen et al., 2007). Nevertheless, inhibition of circulating VEGF reduces vascular permeability and thus tumoral interstitial pressure, permitting easier penetration of the tumor by conventional chemotherapeutic targets (Ferrara, 2005).

A second concern of anti-angiogenesis therapy is the approach to objectify the response to anti-angiogenic drugs. Chan and colleagues found that targeted contrast enhanced micro-ultrasound imaging enables investigators to detect and monitor vascular changes in orthotopic bladder tumors. Therefore, this technique may be useful for direct, noninvasive and in vivo evaluation of angiogenesis inhibitors (Chan et al., 2011). Lassau and colleagues demonstrated that dynamic ultrasound can be used to quantify dynamic changes in tumor vascularity as early as three days after the administration of the anti-angiogenic drug. These changes may be potential surrogate measures of the effectiveness of antiangiogenic therapy, namely by predicting progression-free survival and overall survival (Lassau et al., 2011).

Regarding antilymphangiogenic strategies, numerous compounds that could be used to block lymphangiogenesis already exist, although there is some delay in the translation to the clinic. These act mainly by targeting lymphangiogenic protein tyrosine kinases (Williams et al., 2010) (Table 1) or other indirect regulators of lymphangiogenic events. For instance, rapamycin (sirolimus), a classical immunosuppressant drug used to prevent rejection in organ transplantation, and a known inhibitor of the mTOR (mammalian target of rapamycin) signaling, has demonstrated potent antilymphangiogenic properties (Huber et al., 2007), and may suppress lymphatic metastasis (Kobayashi et al., 2007). mTOR is a member of the phosphoinositide-3-kinase-related kinase family, and is centrally involved in growth regulation, proliferation control and cancer cell metabolism (Rosner et al., 2008). Its inhibition impairs downstream signaling of VEGF-A as well as VEGF-C via mTOR to the ribosomal p70S6 kinase (a regulator of protein translation, and a major substrate of mTOR) in lymphatic endothelial cells (Huber et al., 2007). Other derivative compounds of rapamycin, like everolimus (RAD001) and temsirolimus (Torisel), have also demonstrated anti-tumor properties, namely by inhibiting tumor neovascularization (reviewed in Garcia & Danielpour, 2008). Recently, in patients with lymphangioleiomyomatosis (LAM, a progressive, cystic lung disease in women, which is associated with inappropriate activation of mTOR) sirolimus stabilized lung function, reduced serum VEGF-D levels, and was associated with a reduction in symptoms and improvement in the quality of life (McCormack et al., 2011).

Inhibition of lymphangiogenesis has been shown to block lymphatic metastasis by 50-70% in preclinical animal models, with good safety profiles, which suggests that anti-lymphangiogenic therapy could possibly be used safely in cancer patients, without disrupting normal lymphatic function (reviewed in Holopainen et al., 2011). Optimally, the gold-standard strategy would be the one that could inhibit both angiogenic and lymphangiogenic cascades, in order to compromise the success of haematogenous and lymphogenous dissemination. Some potential compounds are being investigated (reviewed in Boere et al., 2010; reviewed in Cook & Figg, 2010; reviewed in Pinto et al., 2010; reviewed in Stacker & Achen, 2008).

Urothelial bladder carcinoma has experienced very few therapeutic successes, regarding antineovascularization therapy, in the last years. Compounds like bevacizumab (Avastin®), aflibercept (VEGF-Trap, AVE0005), sunitinib malate (Sutent, SU11248), sorafenib (BAY 43-9006), vandetanib (Zactima, ZD6474) and pazopanib (Votrient, GW786034) are being tested in preclinical and clinical trials (reviewed in Pinto et al., 2010) (Table 2).

Bevacizumab, as has been already referred, is a monoclonal antibody that binds and neutralizes VEGF in the serum. Aflibercept is a soluble fusion protein of the human extracellular domains of VEGFR-1 and VEGFR-2, and the Fc portion of human immunoglobulin G. It binds, with a higher affinity than other monoclonal antibodies, to VEGF and additional VEGF-family members, namely VEGF-B and placental growth factor (PlGF). Sunitinib is an oral multi-targeted receptor tyrosine kinase inhibitor, with activity against VEGF receptors and PDGF receptors, among others. Sorafenib is a small, oral molecule that inhibits various targets along the EGFR/MAPK (epidermal growth factor receptor / mitogen-activated protein kinase) signal transduction pathway, and also through VEGFR and PDGFR families. Vandetanib is a tyrosine kinase inhibitor, antagonist of VEGFR and EGFR. Pazopanib is a multitargeted tyrosine kinase inhibitor against VEGF receptors, c-kit, and PDGF receptors (Cook & Figg, 2010).

Gene	Role in lymphatic vessels	Inhibitors available	Effect of pathway inhibition
VEGFR-2	Receptor for the VEGF family of ligands. Can also heterodimerize with VEGFR-3.	Yes	Secreted VEGFR-2 is a naturally occurring inhibitor of lymphatic vessel growth; however, Sorafenib† did not block VEGF-C/D induced tumor lymphangiogenesis.
VEGFR-3	Predominant receptor for VEGF-C and VEGF-D. Transduces survival, proliferation and migration signals.	Yes	Cediranib‡ blocks VEGFR-3 activity and inhibits lymphangiogenesis. Anti-VEGFR-3 antibody prevented tumor lymphangiogenesis with no effect on preexisting vessels.
Tie1	Not critical for lymphatic cell commitment during development, and no ligand has been shown.	None reported.	Tie1 knockout mouse has lymphatic vascular abnormalities that precede the blood vessel phenotype.
Tie2	Receptor for Ang-1 and Ang-2. Appears to control vessel maturation.	Yes	Tie2-/- mice are embryonic lethal due to vascular defects. Inhibition of Ang-2 leads to tumor blood vessel normalization.
EphB4	Expressed on lymphatic capillary vessels. Involved in vascular patterning. Binds to the ephrinB2 ligand.	Yes	Mice expressing a mutant form of ephrinB2 lacking the PDZ binding domain show major lymphatic defects in capillary vessels and collecting vessel valve formation.
FGFR3	The ligands FGF-1 and FGF-2 promote proliferation, migration, and survival of cultured lymphatic endothelial cells. FGFR3 is a direct transcriptional target of Prox1.	Yes	Knockdown of FGFR3 reduced lymphatic endothelial cells' proliferation.
IGF1R	Both of the IGF1R ligands, IGF-1 and IGF-2, significantly stimulated proliferation and migration of primary lymphatic endothelial cells.	Yes	None reported.
PDGFRβ	The ligand PDGF-BB stimulated MAP kinase activity and cell motility of isolated lymphatic endothelial cells.	Yes	None reported.

Gene	Role in lymphatic vessels	Inhibitors available	Effect of pathway inhibition
MET	The ligand for c-Met, hepatocyte growth factor, has lymphangiogenic effect, but it is unclear if c-Met is expressed on lymphatic endothelial cells.	Yes	May be indirect effect.

†Sorafenib inhibits B-Raf, PDGFRb, VEGFR-2 and c-Kit. ‡Cediranib inhibits VEGFR-1, -2, -3, PDGFRb and c-Kit.

Table 1. Protein tyrosine kinases involved in lymphatic biology, and available inhibitors (Tie- tyrosine kinase with immunoglobulin and EGF homology domain; EphB4- ephrin type-B receptor 4) (reprinted by permission from © 2010 BioMed Central Ltd. Originally published in *J. Ang. Res.* 2: 1-13)

Principal investigator / organization	Regimen	Patient population	Phase
Siefker-Radtke/MDACC	Methotrexate + vinblastine + doxorubicin+ cisplatin + bevacizumab	Neoadjuvant (muscle-invasive)	II
Kraft/MUSC	Gemcitabine + cisplatin + bevacizumab → cystectomy → paclitaxel + bevacizumab	Neoadjuvant/adjuvant (muscle-invasive)	II
Hahn/HOG	Gemcitabine + cisplatin + bevacizumab	First-line metastatic	II
Bajorin/MSKCC	Gemcitabine + carboplatin + bevacizumab	First-line metastatic (cisplatin-ineligible)	II
Rosenberg/CALGB	Gemcitabine + cisplatin ± bevacizumab	First-line metastatic	III
Garcia/Cleveland Clinic	Sunitinib	Neoadjuvant (muscle-invasive)	II
Sonpavde/HOG	Gemcitabine + cisplatin + sunitinib	Neoadjuvant (muscle-invasive)	II
Bellmunt	Sunitinib	First-line metastatic (cisplatin-ineligible)	II
Galsky/US Oncology	Gemcitabine + cisplatin + sunitinib	First-line metastatic	II
Hussain/University of Michigan	Sunitinib versus placebo	Maintenance after first-line chemotherapy	II
Gallagher/MSKCC	Sunitinib	Second-line metastatic	II
Milowsky/MSKCC	Gemcitabine + cisplatin + sorafenib	First-line metastatic	II
Kelly/Yale	Gemcitabine + carboplatin + sorafenib	First-line metastatic (cisplatin-ineligible)	II

Principal investigator / organization	Regimen	Patient population	Phase
Sternberg/EORTC	Gemcitabine + carboplatin ± sorafenib	First-line metastatic	II
Dreicer/ECOG	Sorafenib	Second-line metastatic	II
Choueiri/DFCI	Docetaxel ± vandetanib	Second-line metastatic	II
Vaishampayan/Mayo Clinic	Pazopanib	Second-line metastatic	II

MDACC = MD Anderson Cancer Center; MUSC = Medical University of South Carolina; HOG = Hoosier Oncology Group; MSKCC = Memorial Sloan-Kettering Cancer Center; CALGB = Cancer and Leukemia Group B; EORTC = European Organization for Research and Treatment of Cancer; ECOG = Eastern Cooperative Oncology Group; DFCI = Dana-Farber Cancer Institute

Table 2. Selected ongoing or recently completed trials exploring antiangiogenic therapies in urothelial bladder carcinoma (reprinted by permission from © 2010 Elsevier. Originally published in *Commun. Oncol.* 7: 500-504)

4.1 Preclinical studies

In the preclinical scenario, Videira and colleagues studied the effect of bevacizumab on autocrine VEGF stimulation in bladder cancer cell lines, and concluded that, at clinical bevacizumab concentrations, cancer cells compensate the VEGF blockade, by improving the expression of VEGF and related genes. This highlights the need to follow the patient's adaptation response to bevacizumab treatment (Videira et al., 2011). The antiangiogenic treatment of tumours may restore vascular communication and, thereby, normalize flow distribution in tumour vasculature. The use of antiangiogenic drugs leads to improved tumour oxygenation and chemotherapy drug delivery (Pries et al., 2010). However, these mechanisms may be also the cause of malignant dissemination, because tumours elicit evasive resistance. Caution is recommended, due to the divergent effects that VEGF inhibitors can induce on primary tumor growth and metastasis (Loges et al., 2009).

Yoon and colleagues, when exposing six human bladder cancer cell lines to an escalating dose of sunitinib alone or in combination with cisplatin/gemcitabine, demonstrated that sunitinib malate has a potent antitumor effect and may synergistically enhance the known antitumor effect of gemcitabine (Yoon et al, 2011).

The first study with vandetanib in bladder cancer cell lines demonstrated its potential to sensitize tumor cells to cisplatin. At vandetanib concentrations of ≤2microM, the combination with cisplatin was synergistic, especially when given sequentially after cisplatin , and additive with vandetanib followed by cisplatin (Flaig et al., 2009).

Li and colleagues studied the efficacy of pazopanib, both alone and in combination with docetaxel, in bladder cancer cell lines. They demonstrated that single-agent pazopanib has modest activity, but when given in combination with docetaxel, acted synergistically in docetaxel-resistant bladder cancer cells, with the potential of improved toxicity (Li et al., 2001).

Urothelial bladder carcinoma expresses mTOR signaling molecules, providing a rationale for clinical trials evaluating agents targeting this pathway (Tickoo et al., 2011). In fact, some studies using bladder cancer cell lines have demonstrated that sirolimus and related drugs inhibit the growth of cancer cells and decrease their viability (Fechner et al., 2009; Hansel et al., 2010; Pinto-Leite et al., 2009; Schedel et al., 2011). Similar results were obtained when

treating bladder cancer animal models with sirolimus or everolimus (Chiong et al., 2011; Oliveira et al., 2011; Parada et al., 2011; Seager et al., 2009; Vasconcelos-Nóbrega et al., 2011).

4.2 Phase II studies

The results of a phase II trial of cisplatin, gemcitabine, and bevacizumab (CGB) as first-line therapy for metastatic urothelial carcinoma revealed that CGB may improve overall survival — with a median follow-up of 27.2 months, overall survival time was 19.1 months. However, the rate of side effects was high, namely neutropenia, thrombocytopenia, anemia, and deep vein thrombosis/pulmonary embolism (Hahn et al., 2011).

In a phase II trial of gemcitabine, carboplatin, and bevacizumab in patients with advanced/metastatic urothelial carcinoma, Balar and colleagues concluded that addition of bevacizumab does not improve the response rate. However, bevacizumab can be safely added to gemcitabine and carboplatin, because the rate of venous thromboembolisms is similar to the one observed with gemcitabine and carboplatin alone (Balar et al., 2011). Moreover, in a pooled analysis of cancer patients in randomized phase II and III studies, the addition of bevacizumab to chemotherapy did not statistically significantly increase the risk of venous thromboembolisms *versus* chemotherapy alone. Probably, the risk for venous thromboembolisms is driven predominantly by tumor and host factors (Hurwitz et al., 2011). This type of side effect is primarily prevented by using anticoagulants simultaneously with cytotoxic chemotherapy (Riess et al., 2010). However, anticoagulant use during bevacizumab therapy may increase the risk of serious hemorrhage, although it is generally well tolerated (Bartolomeo et al., 2010). This controversial issue is still under scrutiny and more data are needed to clarify the optimal regime to reduce venous thromboembolisms in bladder cancer patients, particularly in those who are being treated with antiangiogenic drugs.

Patients with recurrent or metastatic urothelial carcinoma who had received a prior platinum-containing regimen were entered in a phase II trial with aflibercept as a second-line therapy. Aflibercept was well tolerated, but it had limited single agent activity in platinum-pretreated bladder cancer patients (Twardowski et al., 2009).

In a phase II study of sunitinib in patients with metastatic urothelial cancer designed to assess the efficacy and tolerability of this drug in patients with advanced, previously treated urothelial cancer, anti-tumour responses were observed. However, sunitinib did not achieve the predetermined threshold of ≥20% activity defined by the Response Evaluation Criteria in Solid Tumors, and side effects such as embolic events were reported (Gallagher et al., 2010).

In a multicenter phase II trial with sunitinib as first-line treatment in patients with metastatic urothelial cancer ineligible for cisplatin, on intention-to-treat analysis revealed that 38% of the patients showed partial responses (PRs), and 50% presented with stable disease (SD), the majority more than 3 months. Clinical benefit (PR + SD) was 58%. Median time to progression was 4.8 months and median overall survival 8.1 months (Bellmunt et al., 2011).

In a multicentre phase II trial of sorafenib as second-line therapy in patients with metastatic urothelial carcinoma, there were no objective responses to therapy. The 4-month progression-free survival rate was 9.5%, and the overall survival was 6.8 months (Dreicer et al., 2009).

Choueiri and colleagues conducted a double-blind randomized trial in which patients with metastatic bladder cancer and as many as three previous chemotherapy regimens received intravenous docetaxel with or without vandetanib. The results demonstrated that the

addition of vandetanib to second-line docetaxel did not result in significant improvements in progression-free survival, overall survival or response rates (Choueiri et al., 2011).

The final results of a phase II study of everolimus in metastatic urothelial cell carcinoma have been presented at 2011 ASCO (American Society of Clinical Oncology) Annual Meeting. It was demonstrated that everolimus has clinical activity in patients with advanced urothelial bladder cancer. For the thirty-seven evaluable patients, the median progression-free survival was 3.3 months, and the median overall-survival was 10.5 months. Some side effects possibly related to everolimus were observed, namely anemia, infection, hyperglycemia, lymphopenia, hypophosphatemia and fatigue (Milowsky et al., 2011).

Dovitinib (TKI258) is an oral investigational drug that inhibits angiogenic factors, including FGFR and VEGFR. A multicenter, open-label phase II trial of dovitinib in advanced urothelial carcinoma patients with either mutated or wild-type FGFR3 is currently underway (Milowsky et al., 2011).

4.3 Phase III studies

A randomized double-blinded phase III study comparing gemcitabine, cisplatin, and bevacizumab to gemcitabine, cisplatin, and placebo in patients with advanced urothelial carcinoma is open to enrollment. The primary end point is to compare the overall survival of patients with advanced urothelial carcinoma treated with gemcitabine hydrochloride, cisplatin, and bevacizumab *versus* gemcitabine hydrochloride, cisplatin, and placebo. The secondary end points are to compare the progression-free survival, the objective response rate and the grade 3 and greater toxicities of these regimens in the patients (Cancer and Leukemia Group B, 2011).

5. Conclusion

Bladder cancer represents a significant health problem, and the costliest type of cancer to treat. Although the majority of cases present as non-muscle invasive disease, the recurrence and progression rates are high, which demands for long-term follow-up and repeated interventions. Moreover, patients with advanced tumors treated by neoadjuvant or adjuvant regiments frequently progress and may develop chemotherapy resistance. Therefore, biomarkers of tumour aggressiveness and response to therapy are urgently needed, since the classical formulae based on stage and grade classification are insufficient to characterize bladder cancer. In this sense, angiogenesis, lymphangiogenesis and lymphovascular invasion have been described as surrogate markers of bladder cancer progression, invasion and metastasis, and represent potential fields of intervention. On one hand, the combined analysis of these biological parameters in tumor samples with the classical clinicopathological parameters may improve the individual characterization of bladder cancer, in what concerns to its clinical and prognostic course, and should allow therapeutic adequacy. On the other hand, the knowledge and modulating of biological phenomena related with bladder cancer progression may represent a significant improvement in the development of new drugs and in the pathological response to therapy, which ultimately will lead to an increase in disease-free survival and overall survival rates.

Targeted therapy has caused dramatic changes in the treatment of other types of tumors. However, in bladder cancer setting, clinical trials with molecularly targeted agents have been few in number and largely unsuccessful. Regarding antiangiogenic and

antilymphangiogenic agents, these are still considered an investigational option for urothelial bladder cancer patients, and more results are needed to establish their roles in the treatment armamentarium. Research studies with anti-neovascularization drugs should not only provide effective agents to treat bladder cancer patients, but also predictive biomarkers for response to anti-neovascularization therapy, in order to implement the concept of personalized therapy.

6. Acknowledgements

7. References

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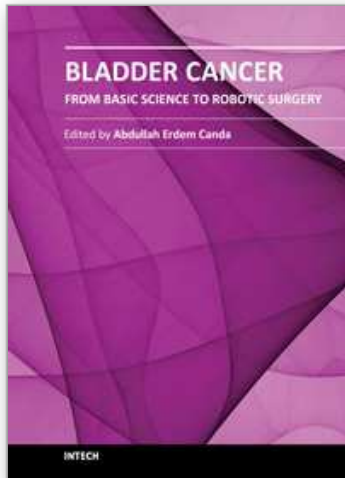
Bladder Cancer - From Basic Science to Robotic Surgery

Edited by Dr. Abdullah Canda

This book is an invaluable source of knowledge on bladder cancer biology, epidemiology, biomarkers, prognostic factors, and clinical presentation and diagnosis. It is also rich with plenty of up-to-date information, in a well-organized and easy to use format, focusing on the treatment of bladder cancer including surgery, chemotherapy, radiation therapy, immunotherapy, and vaccine therapy. These chapters, written by the experts in their fields, include many interesting, demonstrative and colorful pictures, figures, illustrations and tables. Due to its practicality, this book is recommended reading to anyone interested in bladder cancer.

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