PROSPECTS

Targeting Vasculature in Urologic Tumors: Mechanistic and Therapeutic Significance

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Abstract   Recent advances toward understanding the molecular mechanisms regulating cancer initiation and progression provide new insights into the therapeutic value of targeting tumor vascularity by interfering with angiogenic signaling pathways. The functional contribution of key angiogenic factors toward increased vascularity characterizing metastatic tumors and their therapeutic exploitation is considered in three major urologic malignancies, renal, bladder, and prostate cancer. With the realization that the success of the therapeutic efficacy of the various anti-angiogenic approaches for the treatment of urologic tumors has yet to be proven clinically, the challenge remains to select critical angiogenesis pathways that can be targeted for an individual tumor. Here we discuss the major mechanisms that support formation of vasculature in renal, bladder, and prostate tumors and the current results of targeting of specific molecules/ regulators for therapeutic intervention against metastastic disease. J. Cell. Biochem. 103: 691–708, 2008.
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In 2007, there will be an estimated 346,440 new cases diagnosed with urologic cancer in the United States and 54,360 Americans will die from a urologic malignancy (SEER Cancer Statistics Review, http://cancernet.nci.nih.gov/ statistics). This mortality rate is alarmingly high as it translates to one individual dying every 9 min in the US due to a urologic tumor and thus a significant health issue.

Angiogenesis is an essential process in normal physiological functions such as ovarian cycle in female reproductive system [Kaczmarek et al., 2005] and a contributing factor in disease states such as chronic inflammation, arthritis, cancer, and macular degeneration [Folkman, 1995]. During the development of the embryo, mesoderm differentiates into angioblasts; these endothelial cells, not yet organized into a lumen, form primitive vessels toward development of blood vessel network, via vasculogenesis. In the adult, new blood vessels form from pre-existing vasculature, via angiogenesis [Risau, 1997], while malignant conditions induce a hypercoagulable state in their hosts [Nash et al., 2001]. By early 1960s it was evident that tumors could elaborate diffusible substances that induce angiogenesis from the host vasculature [Algira et al., 1945; Greenblatt and Shubick, 1968]. The increased tumor vascularity was originally believed to be vasodilation of the host endothelium in response to metabolic waste products from within the tumor [Folkman, 1995]. A decade later Dr. Folkman's pioneering work identified angiogenesis as a required phenomenon for

tumor growth and metastasis, first defining the potential therapeutic value of agents targeting this process [Folkman, 1995; Folkman, 1971]. Tumor blood vessels exhibit characteristic markers which are not present in normal angiogenic tissues [Ruoslahti, 2002]. After enduring the circulation "journey," metastatic cancer cells can escape out of the endothelial vasculature and in the target tissue via extravasation. How do the metastatic cells signal activating changes in the vascular permeability of blood vessels in target organs? Vascular endothelial growth factor (VEGF) initially identified as potent vascular permeability factor is the lead candidate. Activation of Src family kinases in endothelial cells exposed to VEGF induces disruptions in endothelial cell junctions, facilitating metastatic extravasation. Hypoxia within the tumor mass applies selective pressure promoting the outgrowth of malignant cells, with diminished apoptotic ability. The cellular response to low oxygen tension involves stabilization of a hypoxia-inducible factor-1 (HIF-1) transcriptional complex genes involved in cell survival and invasion.

In this review we discuss the current knowledge on angiogenesis as a contributor to cancer progression, and the clinical exploitation of this knowledge towards molecular targeting of tumor vascularity for the treatment of urologic malignancies.

REGULATION OF ANGIOGENESIS IN TUMOR PROGRESSION

Angiogenic stimuli produced due to metabolic demands of host tissues initiate the angiogenic response [Risau, 1997]. Upon binding to membrane receptors in vascular endothelial cells, a five-step process is triggered: initially the vascular endothelial basement membrane of the parent vessel breaks down, allowing a route for the development of a new capillary sprout, this is followed by migration of endothelial cells through the basement membrane toward the angiogenic stimulus; this leading front of migrating cells is driven by enhanced proliferation of endothelial cells, followed by formation of capillary tubes via organization of the endothelial cells, and a recruitment of periendothelial cells (pericytes) and vascular smooth muscle cells for new capillary stabilization [Cotran et al., 1999; Van Moorselaar and Voest, 2002].

In normal conditions angiogenesis is maintained by an intricate balance between endogenous stimulators of angiogenesis and endogenous inhibitors of angiogenesis (Table I). Additional mechanisms include inhibition of angiogenesis via sequestration of stimulators of angiogenesis in the extracellular matrix (ECM) and changes in the endothelial cell shape, reducing their susceptibility to stimulators [Folkman, 1995]. During tumorigenesis, the angiogenic switch is activated directly via induction of angiogenic growth factors, or indirectly, by recruiting host immune cells that release mediators of angiogenesis [Folkman, 1993]. Circulating endothelial precursor cells (CEP) from the bone marrow also contribute to tumor neovascalurization [Lyden et al., 2001], while tumor cells can recruit new blood vessels due to a network from adjacent endothelial cells [Dameron et al., 1994].

The Major Players: Endogenous Angiogenesis Promoters

Vascular endothelial growth factor. VEGF is the most prominent regulator of physiological angiogenesis [Lonser et al., 2003]. Genetic knockout studies revealed that loss of a single VEGF allele results in embryonic lethality [Ferrara et al., 1996], pointing to a dose-dependent requirement of VEGF for normal vasculature during development [Van Moorselaar and Voest, 2002]. VEGF-A is the protagonist member of the VEGF family that includes VEGF-B, VEGF-C, VEGF-D, and placenta growth factor [Ferrara et al., 2003]. It is a secreted heparin binding protein of four isoforms produced by alternative exon splicing [Orlandi et al., 1996; Jackson et al., 1997] and can be induced by other signaling effectors such as TGF-β, TGF-α, and PDGF [Lara et al., 2004]. VEGF functions are mediated through two tyrosine-kinase receptors, VEGF-R1 (Flt-1) [deVries et al., 1992] and VEGF-R2 (Flk-1 or KDR) [Millauer et al., 1993], in vascular endothelial cells [Hanahan, 1997]. VEGF initially interacts with VEGF-R2 to promote endothelial cell proliferation, migration and vascular permeability, and subsequently with VEGF-R1 to assist the organization of new capillary tubes. Loss of VEGF-R1 impairs the ability of angioblasts to be organized into mature capillaries in vivo [Fong et al., 1995] and VEGF-R2 is of responsible for recruiting

TABLE I. Endogenous Regulator of Angiogenesis

Classification	Angiogengesis stimulators	Angiogensis inhibitors
Growth factor	VEGF	
	PDGF	
	aFGF and bFGF	
	TGF-α and β	TGF-β
	HGF	
	Angiopoetins-1	Angiopoietin-2
	GCSF	
Necrosis factor	TNF-arufa	
Chemokine		Platelet factor 4 (PF4)
Membrane protein	Integrin	
Hormone	Proliferin	2-Methoxyestradiol
Transcription factor	HIF-1, HIF-2	
Cytokine	IL-8	IL-12
		IFN-α and β
Protease	MMPs	
Protease inhibitor		TIMP-1, TIMP-2
		Maspin
		PEX
Plasminogen		Angiostatin
		Plasminogen Kringle 5
Collagen		Endostatin
		Fibronectin fragment
Others	Endothelins	Antithrombin III
		PEDF
		Troponin I
		TSP 1

TSP-1; thrombospondin-1, PEDF; pigment epithelial-derived factor, TIMP; tissue inhibitor of metalloproteinase INF; interferon, IL; interleukin, HIF; hypoxia-inducible factor, MMPs; matrix metalloproteinases VEGF; vascular endothelial growth factor, TGF; transforming growth factor, PDGF; platelet-derived growth factor HGF; hepatocyte growth factor, FGF; fibroblast growth factors: acidic (aFGF) and basic (bFGF) GCSF; Granulocyte colony-stimulating factor.

cells in the developing vasculature [Barleon, 1996]; induction of matrix metalloproteinases (MMPs) [Hiratsuka et al., 2002] and secretion of additional growth factors from the developing endothelium (Fig. 1) [LeCouter et al., 2003].

HIF-α (Hypoxic-inducible factor-α). Tissue hypoxia is associated with rapid tumor growth and VEGF upregulation through HIF-1, which increases transcription and the stability of VEGF mRNA [Shweiki et al., 1992; Semenza, 1996]. HIF-1 is a ubiquitous bHLH/PAS (basic helix-loop-helix/Per-Arnt-Sim homology) transcription factor that is composed of the two basic helix-loop-helix PAS proteins, HIF-1α and HIF-1β [Wang et al., 1995]. Three α subunits, such as HIF-1 α, HIF-2 α, and HIF-3 α, have been identified. Under normoxia, prolyl hydroxylation of the HIF-α subunit is enhanced, and mediates interaction with von Hippel-Lindau protein (pVHL), E3 ubiquitin ligase that then undergoes proteosomal degradation. At low oxygen levels, prolyl hydroxylation of HIF is inhibited, escaping from degradation through pVHL. HIF-α will translocate into the nucleus to form a heterodimeric complex with HIF-β and subsequently stimulate gene transcription [Schofield and Ratcliffe,

2004]. As illustrated in Figure 2, HIF induces transcriptional activation of genes regulating key processes in tumor progression, such as angiogenesis (Ang-2), glucose metabolism, (glucose transporter 1), adhesion (E-cadherin, Vimentin), migration (TGF-α, c-Met), proteolysis (Cathepsin D, uPAR, MMP2), and pH (CA IX; carbon anhydorase 9) [Pouyssegur et al., 2006]. Tumors that exhibit abundant HIF-1 stabilization have a greater likelihood of developing metastatic relapse and shorter survival with a subset of HIF target genes acting as mediators of metastastic progression [Semenza, 2000]. HIF-1 induces the chemokine receptor CXCR4 in renal cell carcinoma cells, which promotes organ-specific metastatic dissemination [Staller et al., 2003]. Moreover a mutation in von Hippel-Lindau (VHL) suppressor gene results in VHL hereditary cancer syndrome leading to sporadic clear cell renal cell cancers (CCRCC) [Kaelin, 2002].

Angiopoietins. Angiopoietins are ligands for TIE-2 receptors located on endothelial cells. Angiopoietin-1 (Ang1) interacts with the TIE-2 receptor to recruit periendothelial cells, such as pericytes and vascular smooth muscle cells, for stabilization of the new vasculature [Luo et al., 1997]. Ang2 interacts with TIE-2 to increase
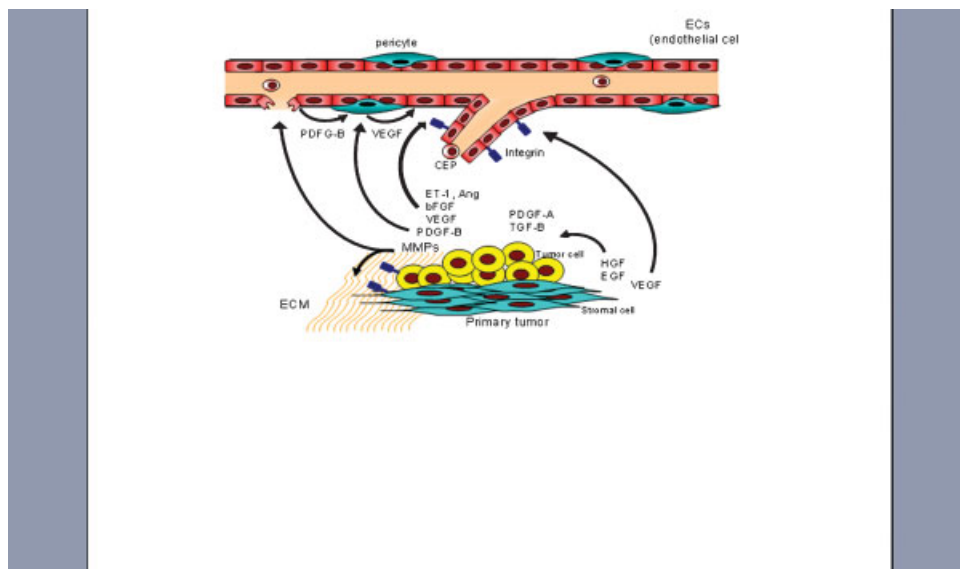
Fig. 1. Mechanistic interaction between VEGF and FGF signaling in the tumor microenvironment. Tumor epithelial cells produce angiogenic factors such as VEGF, bFGF, ETs, and angiopoietins; BEGF is also secreted by stromal cells. Integrin (a(v)b3) is upregulated in growth-factor activated endothelial cells. MMPs mediate degradation of the ECM and cooperate to enhance angiogenesis and vascular remodeling. Tumor epithe-lial cells communicate with the microenvironment via growth factor signaling interactions: PDGF and TGF-β by tumor cells and EGF and HGF by stromal cells. PDGF-B produced by both endothelial and tumor epithelial cells, stimulates VEGF expression by pericytes to enhance endothelial cell survival. CEP cells from the bone marrow also contribute to new blood vessel formation.

vascular permeability, enhancing susceptibility to angiogenesis stimulators in the presence of VEGF, while in its absence sensitizing the endothelium to endogenous inhibitors [Davis et al., 1996]. The pattern of VEGF-dependent signaling of TIE-2 receptors dictates angiogenesis [Puri et al., 1995; Sato et al., 1995]; as suggested by embryonic lethality of TIE-2
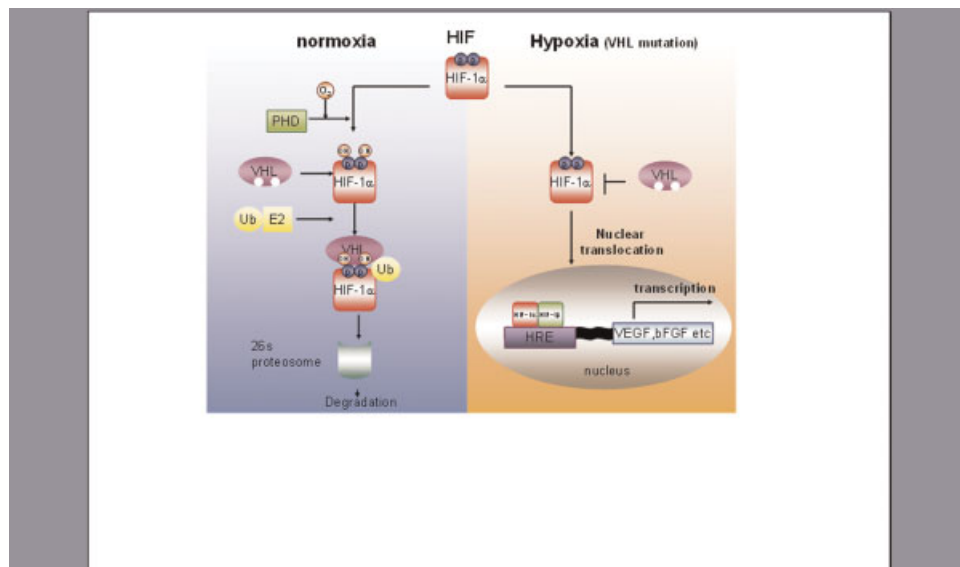
Fig. 2. Hypoxia takes control of tumor angiogenic responses. HIF is a heterodimeric transcription factor consisting of α and β subunits. Under normoixic conditions, prolyl-hydroxylase domain (PHD) protein hydroxylates the two proline residues (Pro402 and Pro564) in the oxygen-dependent degradation domain (ODDD) of HIF-1 α. Hydroxylation of these proline residues allows HIF-1α to interact with von Hippel Lindau tumor suppressor protein (VHL), leading to rapid ubiquitilation and degradation of HIF-1α. HIF1-α escape from the VHL mediated degradation allows binding with HIF-1 β at the nuclear response element, thus activating transcription of target genes including VEGF and bFGF. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

knockout mice due to lack of brain capillary sprouting [Puri et al., 1995].

Fibroblast growth factors. Basic fibroblast growth factor (bFGF) was the first molecule to be identified as a pro-angiogenic agent [Shing et al., 1984]. bFGF binds tightly to heparin sulfate proteoglycans (HSPGs), on the cell surface and ECM [Vlodavsky et al., 1991], and this complex subsequently stabilizes bFGF from heat and proteolysis [Flaumenhaft et al., 1990]. FGFs interact with FGF-receptors (FGFR-1 or FGFR-2), leading to endothelial cell proliferation [Cross and Claesson-Welsh, 2001], degradation of the, stimulation of chemokines toward endothelial cell migration, regulation of integrin and cadherin expression, and modulation of cell–cell interactions [Presta et al., 2005]. Engaged in a dynamic cross-talk, FGF, can directly upregulate VEGF expression in endothelial cells [Tille et al., 2001]. A synergistic action between VEGF and FGF generates a significant angiogenic response in target cells [Xue and Greisler, 2002], and consequently VEGF targeting antibodies and VEGFR-2 antagonists inhibit angiogenesis mediated by both VEGF and FGF [Cross and Claesson-Welsh, 2001] (Fig. 1).

Matrix metalloproteinases. MMPs is a family consisting of 16 members of zinc-dependent proteases [Kleiner and Stetler-Stevenson, 1999] that mediate ECM degradation. The four major subgroups of this protease family are gelatinases, collagenases, stromelysins, and membrane associated proteases [Jiang and Muschel, 2002]. Most MMPs released in their inactive state are cleaved to activation by other MMPs or other serine proteases such as plasmin and urokinase-type plasminogen activator [Birkedal-Hansen et al., 1993; Goetzl et al., 1996]. Tissue inhibitors of matrix metalloproteinases (TIMPs) provide an additional level of regulation for MMP activation [Birkedal-Hansen et al., 1993]. ECM undergoes constant remodeling in normal homeostasis and MMPs function to remove proteins from the basement membrane during this remodeling [Kleiner and Stetler-Stevenson, 1993]. Their cooperation targets degradation of basement membrane of the vascular endothelium and ECM, thus creating a passageway in these physical barriers toward new capillary formation [Kleiner and Stetler-Stevenson, 1999].

Integrins. Integrins are transmembrane proteins that serve a role as primary mediators of cell-ECM interactions that are functionally involved in determining tumor angiogenic response during cancer progression to metastatic disease. Integrins (heterodimers containing two distinct chains, α and β subunits), recognize the major adhesive ECM components, fibronectin and laminin, toward regulation of cell proliferation, cell survival, anoikis, and migration [Giancotti and Ruoslahti, 1999; Goel and Languino, 2004]. Angiogenic growth factors such as VEGF and pFGF can exert a profound positive effect on the activity and expression of several integrins, such as $\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_v\beta_1$, $\alpha_3\beta_1$, $\alpha_3\beta_1$, $\alpha_6\beta_1$, $\alpha_6\beta_4$ [Klein et al., 1993]. Additional angiogogenic factors, such as class 3 semaphorins (SEMA3 proteins), control vascular remodeling via targeting integrin [Serini et al., 2003]. Increased expression of integrin $\alpha_v\beta_3$ is detected in growth factor-activated endothelial cells in tumor blood vessels and granulation tissue [Enenstein et al., 1992; Brooks et al., 1994], while very low expression was detected in resting blood vessels [Arap et al., 1998]. Expression of the $\alpha_v\beta_3$ in tumor endothelium correlated with the aggressive phenotype in neuroblastoma [Erdreich-Epstein et al., 2000]. Moreover, blocking integrin $\alpha_v\beta_3$ with monoclonal antibody (Mab) mediated endothelial cell apoptosis and inhibited blood vessel formation [Enenstein et al., 1992], implicating its functional significance in angiogenesis. Consequently, $\alpha_v\beta_3$ integrin represents an attractive tumor vasculature target [Hood et al., 2002; Ruoslahti, 2002]. Nanoparticles containing agents linked with an anti-$\alpha_v\beta_3$ antibody [Sipkins et al., 1998] or bacteriophage with $\alpha_v\beta_3$ binding RGD peptide [Arap et al., 1998] effectively targeted tumor vasculature. Integrins also contribute to signal transduction from the extracelluar environment to the intracellular network mediated by integrin-activated signaling molecules, such as focal adhesion keinase (FAK), phosphatidylinositol 3-kinase (PI 3-kinase), and members of the extracellular signal-regulated kinase 1 and 2/mitogen activated protein (ERK1 and 2/MAP) kinase family to regulate cell proliferation, migration, and apoptosis of tumor and endothelial cells [Fornaro et al., 2001; Nikolopoulos et al., 2004].

Endothelins. Endothelins, a class of proteins involved in vasoconstriction, have also been linked to angiogenesis. Epithelial cells produce three types of endothelins, ET-1, 2, and 3 [Kopetz et al., 2002] that interact with two

receptors, ET-A and ET-B. ET-3 stimulates ET-B receptor to induce endothelial cell growth and blood vessel formation [Lara et al., 2004]. A dynamic cross-talk between ET-1 and ET-A receptor promotes VEGF release [Goligorsky et al., 1999] leading to a strong angiogenic response [Salani et al., 2000].

TARGETING TUMOR VASCULARITY: THERAPEUTIC SIGNIFICANCE

Several angiogenesis-targeting approaches are currently evaluated in clinical trials for their therapeutic efficacy and long-term clinical benefits, while others are mechanistically exploited toward the development of novel therapeutic modalities for advanced urologic malignancies. The molecular platforms for such approaches are considered in Figure 3.

Kidney Cancer

The clinical problem. Renal cell carcinoma accounts for more than 30,000 new cases of cancer and 12,000 deaths in the USA annually. The incidence of RCC has increased by >30% in the last decade [Motzer et al., 1996] and the annual mortality-to-incidence ratio for RCC is significantly higher than for other urological tumors. In spite of recent improve-

ment in imaging for early diagnosis, 25–30% of patients have metastasis at presentation; the 5 year survival rate for patients with metastasis is less than 10% and the overall 5-year survival rate is 60% [Kirkali et al., 2001; Schrader et al., 2006]. The current therapeutic responses in metastatic RCC patients are poor. Indeed combination regimes of recombinant cytokines with interferon or interleukin-2, currently presumed to be the most effective therapy, exhibit modest response rate of 10–20% [Schrader et al., 2006]. The options for those patients failing cytokine-based therapy are limited, hence the emerging clinical challenge. VHL syndrome is an autosomal dominant neoplasia syndrome that results from a germline mutation in the *VHL* gene [Crossey et al., 1994]. This gene mutation is found in 34–57% of clear cell RCC tumors [Rini, 2005; Rini and Small, 2005]; moreover, VHL gene inactivation through methylation of VHL gene is reported in 5–19% of RCC tumors [Rini and Small, 2005]. VHL inactivation in the low-grade and smaller (pT1) tumors, suggests that VHL inactivation may be involved in tumor initiation [Schrader et al., 2006]. Since VHL specifically targets the hydroxylated HIF-1 α subunit and mediates proteasomal degradation, mutations or inactivation of VHL genes that inhibit HIF-1α
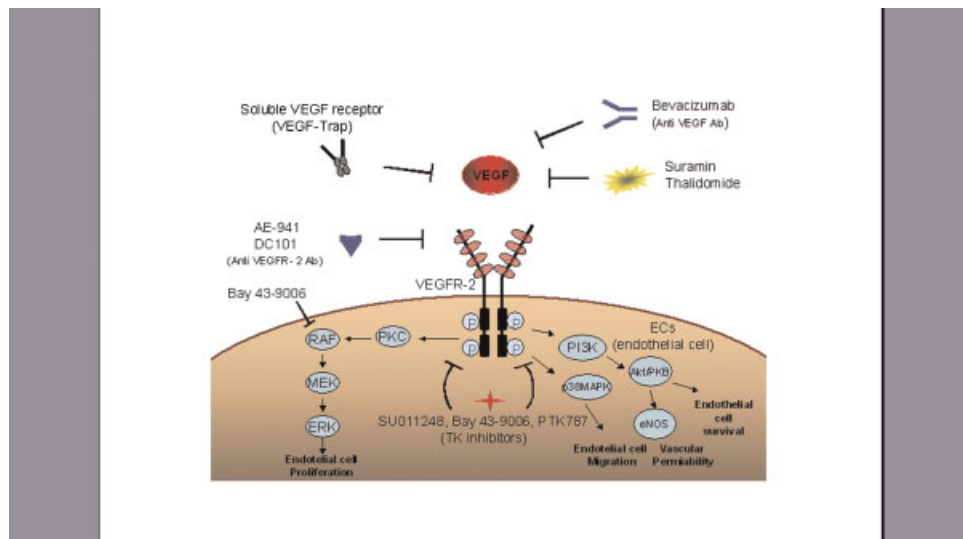

Fig. 3. Targeting tumor vascularity: VEGF inhibitors take central stage. VEGF binding to its receptor mediates dimerization of VEGFR and induces autophosphorylation of RTKs, which initiate activation of several downstream pathways, regulating endothelial cell proliferation (via PKC-RAF-MEK-ERK), survival (via PI3K-AKT/PKB), migration, (via p38-MAPK) and vasculature permeability (via PI3K-AKT/PKB-eNOS). Bevacizumab, a neutralizaing antibody against VEGF directly targets VEGF; Thalidomide inhibits VEGF transcription; Suramin is a nonspecific inhibitor of multiple growth factor signalig pathways; Chimeric soluble receptor (VEGF-Trap) interferes with VEGF binding to its receptor; AE-941 and DC101 are anti-VEGFR-2 antibody abrogating VEGF binding to its receptor. SU011248, Bay 43-9006, PTK787 inhibit phosphorylation of VEGF receptor. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

degradation and enhance transcription of hypoxia-related genes including VEGF [Rini, 2005].

More than 90% of hypervascular renal cell tumors express elevated VEGF mRNA compared to normal kidney [Hemmerlein et al., 2001]. Strong correlations have also been documented between VEGF up-regulation in RCC tumors with nuclear grade and TNM stage [Paradis et al., 2000; Lee et al., 2001] as well as a poor prognostic outcome [Paradis et al., 2000]. The majority of RCC tumors harboring a VHL mutation express HIF that significantly correlates with elevated VEGF [Na et al., 2003]. This evidence easily supports the concept that VHL inactivation in RCC is causally involved in promoting tumor angiogenesis via VEGF upregulation and consequently directly blocking VEGF, becomes an attractive therapeutic strategy for managing advanced RCC.

Specific single inhibitors such as TNP-470 (anti-VEGF) [Stadler et al., 1999] and SU5416 (Tyrorsine kinase inhibitor) [Kuenen et al., 2003] have failed to provide a therapeutic benefit; however, targeting multiple signaling pathways with combination srtategies bevasizumab+ erlotinib(PR:40%) [Hainsworth et al., 2004] or with the new tyrosine kinase inhibitor SU011248(PR:40%) [Mendel et al., 2003] have successfully delivered significant therapeutic responses. The leading anti-angiogenic molecules that had been investigated in clinical trials for RCC treatment are discussed.

Targeting Angiogenesis in RCC: Therapeutic Outcomes and Clinical Promise

Thalidomide therapy. Thalidomide is an anti-inflammatory and immunosuppressive agent that blocks angiogenesis through inhibiting growth factor, such as bFGF and VEGF, down regulate cytokines, such as tumor necrosis factor-α (TNF-α), modifies cell adhesion molecule expression, and promotes natural killer and T-cell activity [Rini and Small, 2005]. Clinical trials assessing the therapeutic efficacy of thalidomide as a single-agent therapy, revealed an overall response rate of 5.2% (0–16.7%) [Rini and Small, 2005]. Phase II studies with combination regimes of interferon-α or interferon-α plus capecitabine with thalidomide, yielded a distinct objective response of 20% in both trials [Hernberg et al., 2003; Amato and Rawat, 2006]. Furthermore, combination

therapy of interleukin-2 plus thalidomide (in a phase II study) resulted in 41% response, with further modification enhancement by macrophage colony-simulating hormone [Amato et al., 2006]. Combination of bevasizumab with thalidomide failed to yield any therapeutic benefit over monotherapy [Elaraj et al., 2004].

Suramin. Suramin is a polysulphonate naphthylurea which has a potential to inhibit binding of a number of growth factors to their receptors, which act not only for tumor epithelial cells but also endothelial cells [Stein, 1993]. A phase II trial of suramin was conducted in 26 patients with advanced renal cell carcinoma, but the therapeutic responses were minimal (minor response observed for five patients for >3 months), while the toxic side effects were serious including an immune-mediated thrombocytopenia and Staphylococcus sepsis without neutropenia [Motzer et al., 1992]. Another suramin-based phase II clinical trial in 22 patients with advanced renal cell carcinoma revealed no objective response [Schroder et al., 2001], challenging its therapeutic value in RCC.

Bevacizumab. Bevacizumab is a recombinant human Mab which binds and neutilizes all the active isoform of the VEGF. In a preclinical model, Bevacizumab inhibits VEGF-induced proliferation of endotherial cells, leading to inhibition of tumor growth in a number of primary xenograft and metastatic models [Presta et al., 1997; Mordenti et al., 1999; Hu et al., 2002]. A Phase II clinical trial to evaluate the therapeutic efficacy of bevasizumab in RCC, resulted in a partial response rate (10% in high dose group) and prolonged time to disease progression [Yang et al., 2003]. An equally partial response was achieved by the combination of bevacizumab with the EGF-R antagonist (erlotinib), in 57 patients with metastatic RCC [Hainsworth et al., 2004].

SU011248. SU011248 is a multi-targeted receptor tyrosine kinase inhibitor which has reported to potently inhibit PDGF receptors α and β, VEGFR-1 and -2, KIT, and FLT3 (fms-related tyrosine kinase/Flk2/Stk-2) [Motzer et al., 2004, 2006]. A recently conducted A phase II trial using SU011248 in advanced 63 RCC patients (failing to respond initial cytokine treatment), demonstrated a partial response achieved in 25 patients (40%), with major toxicity including lymphopenia, elevated lipase, and fatigue/asthenia [Mendel et al., 2003].

PTK 787. PTK 787 is also a tyrosin kinase inhibitor of VEGFR-1, VEGFR-2, and PDGFR-b. In vivo experiment using murine renal carcinoma model demonstrated that oral administration of PTK787 (50 mg/kg) to mice significantly inhibit the growth of the primary tumor and metastasis compare to control group [Wood et al., 2000]. Recently George et al. reported a phase I/II clinical trial in patients with metastatic RCC. The results indicate a 5% of partial response rate, and 15% of minor response (25–50% shrinkage) rate with significant differences in vascular permeability and reduction in blood flow [George et al., 2003].

Bay 43-9006. Bay 43-9006 is an orally bioavailable bi-aryl urea Raf kinase inhibitor that directly inhibits VEGFR-2, VEGFR-3, and PDGFR-b in a Ras-dependent human tumor xenograft model [Lyons et al., 2001; Wilhelm et al., 2003]. In the clinical setting, a phase II randomized study of Bay 43-9006, in refractory solid tumors including 202 metastatic RCC patients showed a positive therapeutic response [Ratain et al., 2006]. With a large number of patients (42%) achieving an initial response (tumor shrinkage), and a significantly longer median progression-free survival (PFS) in response to sorafenib treatment compared to the placebo [Ratain et al., 2006]. An interim phase III analysis indicated that Sorafenib monotherapy results in a significant increase in progression-free survival in patients with advanced RCC, delivering promising therapeutic value.

SU5416. SU5416 is a small organic molecule that blocks VEGF-mediated signaling by interfering Flk-1, a transmembrane tyrosine kinase. In preclinical studies, SU5416 inhibited endothelial cell proliferation and neovasculization [Fong et al., 1999]. In a phase II trial of SU5416 conducted in 29 patients with advanced or metastatic RCC, no objective therapeutic response was observed [Kuenen et al., 2003]. Furthermore, the combination approach of SU5416 with interferon-α, yielded no objective response, although half of the patient population demonstrated stable disease [Lara et al., 2003].

VEGF-trap. VEGF trap is a decoy receptor created from the combination of VEGFR-1 immunoglobulin (Ig) domain2 and VEGFR-2 Ig domain 3 attached to the human IgG1. This artificial protein binds VEGF with 100-fold increase affinity compare to bevasizumab [Rini, 2005]. In vitro study demonstrated that VEGF-trap efficiently inhibited phosphorylation of VEGFR-2 and VEGF-induced endothelial cell proliferation. In vivo mouse model, VEGF-trap significantly inhibits the growth and vascularity of various tumors [Holash et al., 2002]. A phase-I clinical trial using VEGF-trap involving 33 patients with refractory solid tumors, including nine metastatic RCC patients (escalating dose cohorts, 0.025–0.8 mg/kg), revealed no positive response [Dupont et al., 2004].

AE-941. The identification of anti-angiogenic and anti-tumor growth properties associated with the shark cartilage led to the synthesis of several novel compounds. AE-941 (Neovastat) is a pure shark cartilage compound that selectively inhibits -2, -9, and -12 and competes for the binding of VEGF to its receptor (VEGFR-2), resulting in endothelial cell apoptosis [Batist et al., 2002]. A phase-I trial of AE-941 was performed in 144 patients with refractory solid tumors, including patients with metastatic RCC. As shown on Table II, treatment with AE-941 (either an escalated or high single dose), resulted in an objective response in a mere 14% of patients [Batist et al., 2002].

Prostate Cancer: The Leading Tumor in Males

Prostate cancer is the most common malignancy and is the second leading cause of cancer death in males [Weir et al., 2003]. The initial standard therapy for locally advanced or metastatic disease is androgen deprivation therapy via surgical or medical castration. The initial therapeutic response to the antiandrogen-therapy, is only brief (8 month-3 years), and prostate cancer patients become refractory to additional treatment, as tumors eventually relapse to an androgen independent state [Daneshgari and Crawford, 1993] and development of hormone refractory prostate cancer (HRPC). Docetaxel was shown to prolong survival in patients with HRPC [Petrylak et al., 2004; Pronzato and Rondini, 2005], but chemotherapeutic strategies for effective disease control are still required. The significance of angiogenesis in human prostate cancer progression has been firmly established [Borre et al., 1998; Bono et al., 2002]. Several independent studies documented a significant correlation between microvessel density (MVD) with Gleason score, pathological stage, and patient survival [Borre et al., 1998; Bono et al., 2002]. Furthermore

TABLE II. Summary of Clinical Trials

Drug	Cancer	Objective Response	References
Anti VEGF			
Suramin	RCC	0–4%	Motzer et al. [1992, 1996, 2004, 2006]
			Schroder et al. [2001]
	HRPC	7–15%	Small et al. [1999, 2000, 2002]
Plus hydrocortisone	HRPC	33%(PSA)	Small et al. [1999, 2000, 2002]
TNP-470	RCC	0%	Stadler et al. [1999, 2004]
	HRPC	0%	Logothetis et al. [2001]
Thalidomide	RCC	0–16.7%	Rini et al. [2005]
	HRPC	37.5%(PSA)	Drake et al. [2003]
Plus Interferon-α	RCC	20%	Hernberg et al. [2003]
Interferon-α+capecitabine	RCC	20%	Amato [2006]
IL-2	RCC	CR 7%, PR 33%	Amato et al. [2006]
Paclitaxel/doxorubicin	HRPC	82%(PSA)	Amato et al. [2006]
Docetaxel	HRPC	50%(PSA)	Leonard et al. [2003]
GM-CSF	HRPC	23%(PSA)	Dreicer et al. [2005]
Bevacizumab	RCC	0%	Elaraj et al. [2004]
2-methoxyestradiol	HRPC	0%	Sweeney et al. [2005]
Neutralizing Ab			
Bevacizumab	RCC	10%	Yang et al. [2003]
Plus Docetaxel+estramustine	HRPC	77%(PSA)	Picus et al. [2003]
Erlotinib	RCC	25%	Hainsworth et al. [2004]
Cisplatin+gemcitabin	BT	on going	Sternberg et al. [1988]
Paclitaxel	BT	on going	Sternberg et al. [1988]
Docetaxel+prednisone+thalidomide	HRPC	on going	Schofield and Ratdiffe [2004]
Docetaxel+prednisone	HRPC	on going	Schofield and Ratcliffe [2004]
TK inhibitor			
SU5416	RCC	0%	Kauenen et al. [2003]
	HRPC	0%	Stadler et al. [1999, 2004]
PTK 787	RCC	5.0%	George et al. [2003]
SU011248	RCC	40%	Mendel et al. [2003]
Novel approach			
AE-941	RCC	14%(high dose)	Batist et al. [2002]
VEGF Trap	RCC	0%	Dupont et al. [2004]

RCC, renal cell carcinoma; HRPC, hormone-refractory prostate cancer; BT, bladder tumor (PSA), More than 50% reduction of serum prostate-specific antigen (PSA) is achieved after treatment.

VEGF levels are significantly increased in prostate tumors (relative to normal tissue), an upregulation that directly correlates with tumor stage, differentiation and disease specific survival. A strong expression of VEGF is detected in neuroendocrine-differentiated (NE) tumor cells [Borre et al., 2000]. Serum VEGF levels are significantly higher in metastatic prostate cancer patients compared to localized disease [Duque et al., 1999]. HIF, a key mediator of VEGF expression, is highly expressed in prostate cancer, compared to normal and benign prostate tissue [Du et al., 2003]. HIF-1α mutations (homozygous P582S) in the oxygen-dependent degradation domain of HIF-1α, which mediates continuous activation of HIF, are significantly associated with increased risk for prostate cancer [Orr-Urtreger et al., 2007], indicating the importance of HIF-1α mediated VEGF regulation in prostate tumorigenesis (Fig. 2).

Targeting prostate tumor vascularity: Is there a therapeutic value? Several clinical trials using potent anti-angiogenic drugs, TNP-470 [Logothetis et al., 2001], 2-methoxyestradiol [Sweeney et al., 2005] and SU5416 [Stadler et al., 2004] failed to deliver any positive therapeutic efficacy in prostate cancer patients with hormone refractory disease. Disappointing as these outcomes might be, a series of new angiogenesis targeted approaches have emerged from the laboratory to the clinic, promising considerable clinical benefit.

Selective vasculature targeting. Recent work by Ruoslahti et al. identified the peptides that specifically recognize the vasculature in the prostate through screening phage-displayed peptide libraries. Intravenous injection of chimeric peptide consisting of the SMSIARL homing peptide, linked to a proapoptotic peptide, induced prostate tumor destruction and delayed cancer development in TRAMP mice [Arap et al., 2002].

Doxazosin. Experimental evidence indicates that the quinazoline-based compounds, such as doxazosin and terazosin (clinically used for treatment of hypertension and BPH), may also function in inducing prostate smooth

muscle cell death via apoptotic pathways involving activation of TGF-β1-mediated apoptotic signaling and inhibition of Akt [Garrison and Kyprianou, 2006]. Growing evidence also implicates anoikis (ECM detachment-induced apoptosis) in prostate tumor epithelial and endothelial cells in response to quinazolines [Rennebeck et al., 2005], potentially via targeting VEGF-mediated angiogenic response [Garrison and Kyprianou, 2006].

Suramin. The therapeutic efficacy of suramin was analyzed in a large population of patients (390) with advanced prostate cancer with a randomized dosing. The objective response rate was 9–15%, while the PSA response rates were 24–34% [Small et al., 2002]. In a randomized phase-III trial of suramin in combination with hydrocortisone conducted in 460 hormone-refractory prostate cancer patients, patients receiving suramin had higher PSA response rate, compared to the hydrocortisone arm [Small et al., 2000] (Table II); but still, serious limitations are associated with the clinical efficacy of this agent.

Thalidomide. Preclinical experiments revealed that treatment with N-substituted thalidomide, a potent angiogenesis inhibitor, analog CPS11, (targeting platelet-derived growth factor alpha) led to 90% inhibition of tumor growth and 64% reduction in tumor vascularity of PC-3 human prostate xenografts [Ng et al., 2004]. An open trial of the efficacy and safety of thalidomide in 20 patients with androgen-independent prostate cancer resulted in significantly reduction in PSA levels in 35% of patients [Drake et al., 2003]. Prostate cancer patients with hormone refractory-disease exhibited a relatively good response to a combination treatment of docetaxel and thalidomide, by achieving a significantly improved median overall survival (28.9 months) compared to docetaxel-alone (14.7 months) [Leonard et al., 2003]. In a triple-combination strategy of paclitaxel and doxorubicin with thalidomide, 9 out of 12 patients showed a 50% decrease in PSA [Amato and Sarao, 2006]. Combinational approaches using granular-macrophage colony-stimulating factor (GM-CSF) have been reported with some therapeutic promise. GM-CSF regulates the dendritic cell and tumor-specific cytokine T-cell mediated response [Small et al., 1999], and clinically GM-CSF in combination with thalidomide results in a significant PSA response (23%) [Dreicer et al., 2005].

Bevasizumab. A phase-II Cancer and Leukemia Group B (CALGB) 90006 trial of bevasizumab in combination of docetaxel, and estramustine with a premedication with decadron in chemotherapy-naïve HRPC delivered promise (with the majority of patients achieving >50% PSA reduction) [Picus et al., 2003] and led to two clinical trials. The ongoing clinical trials are an NCI-phase-II study of a four-drug combination strategy of docetaxel, prednisone, thalidomide, and bevacizumab in men with chemotherapy-naïve progressive hormone-refractory prostate cancer (ClinicalTrials.gov, http://clinicaltrials.gov/ct/show); and a CALGB phase III, double-blind, placebo-controlled trial of docetaxel plus prednisone with or without bevacizumab (ClinicalTrials.gov, http://clinicaltrials.gov/ct/show).

SU5416. The initial in vivo anti-tumor effect of SU5416, a small molecule VEGFR2 inhibitor, was determined in the transgenic adenocarcinoma of the mouse prostate (TRAMP) mouse model. Administration of SU5416 to mice (16–22 weeks of age; highly expressing VEGFR-2), resulted in a dramatic decrease in tumor-associated mean vessel density and increased apoptosis [Huss et al., 2003]. Further preclinical studies using PC-3 human prostate cancer xenografts, demonstrated that a combination regime of the VEGFR2 inhibitor, SU5416 with another potent anti-angiogenesis agent, endostatin, led to a significant delay in the onset of tumor progression [Abdollahi et al., 2003].

Bladder Cancer: The Clinical Problem

Bladder cancer is the second most common malignancy in the genitourinary tract and fifth most common solid cancer in the United States and the incidence continuously increasing in recent years. Fifty-four thoudand patients are diagnosed and 12,000 patients die of bladder cancer annually [Greenlee et al., 2000]. The majority of bladder transitional cell carcinoma (TCC) of the bladder cases is of the superficial type (70%), while the rest are invasive and highly metastatic tumors [Beecken et al., 2005]. The standard treatment for operable invasive TCC is radical cystectomy and bladder tumors are initially sensitive to conventional chemotherapy approaches; the median survival in response to methotrexate, vinblastine, adriamycin, and cisplatin regimen however, is only 13–15 months due to emergence of

chemoresistance [Sternberg et al., 1988]. Distinct angiogenic pathways differentiate superficial bladder tumors from invasive tumors. Superficial bladder tumor consists of a papillary morphology with an integrated branching vascular tree, expressing VEGF. In contrast, invasive solid bladder tumors contain a disorganized vascular network with necrotic regions, primarily expressing bFGF [O'Brien et al., 1997]. Elevated VEGF levels correlate with early recurrence and progression of superficial bladder tumors [O'Brien et al., 1995; Crew et al., 1996, 1997], as well as resistance to chemotherapy in patients with advanced disease [Slaton et al., 2004]. Pre-treatment of TCC cells with anti-sense VEGF increased cell sensitivity to conventional chemotherapeutic agents such as Mitomycin C, providing a promising possibility for adjuvant therapy [Krause et al., 2005]. Since bFGF also contributes to bladder cancer progression via its ECM involvement, this player becomes a prime candidate for therapeutic targeting [O'Brien et al., 1997; Guo et al., 2003].

Therapeutic efficacy of anti-angiogenic approaches in bladder cancer

TNP-470. TNP-470 is a synthetic analog of fumagillin which blocks the growth of new blood vessels by inhibiting methionine amino peptidase, an enzyme critically important for endothelial cell proliferation [Ingber et al., 1990]. The therapeutic efficacy of TNP-470 in invasive bladder cancer has been investigated in mouse models using KK-47 and MGH-U1 TCC cancer cell xenografts. Treatment with TNP-470 significantly suppressed tumor growth and vascularity, with limited toxicity [Beecken et al., 2000].

Suramin. Suramin, a potent antagonist of VEGF, is a polysulphonate naphthylurea, exerts distinct anti-tumor effects in mouse models of tumorigenesis. Suramin administration in rats bearing N-methyl-N-nitrosurea (MNU)-induced bladder tumors (twice a week for 18 weeks) resulted in 0–10% of treated group developing papillary bladder tumors [Bikfalvi et al., 1991]. A dose-escalation phase-I study with suramin in a small number of patients with a history of recurrent superficial bladder cancer (n = 12), resulted in a considerable suppression of urinary VEGF [Graham et al., 1995], but failed to deliver a therapeutic effect.

VEGFR monoclonal antibody DC101. Preclinical-testing of the synergetic effect of VEGF blockade with other chemotherapeutic agents in bladder cancer promises enhanced efficacy. Combination of anti-VEGFR Mab therapy with Paclitaxel chemotherapy in TCC mouse model, resulted in a significant tumor regression and inhibition of lymph node metastasis, driven by endothelial cell apoptosis [Ord et al., 2005].

Bevacizumab. Bevacizumab is another Mab designed to target all VEGF isoforms, with an established antiproliferative profile in various cancers [Inoue et al., 2000]. Several ongoing phase-II clinical trials, including the neoajuvant Bevacizumab with cisplatin and gemcitabine followed by radical cystectomy, adjuvant bevacizumab, and paclitaxel in patients with muscle-invasive, resectable TCC, and Bevacizumab, in combination with cisplatin and gemcitabine in patients with metastatic TCC are ongoing, in anticipation of successful outcomes [de Gramont and Van Cutsem, 2005].

CONCLUSION

Recent advances toward a better understanding of the molecular mechanisms underlying urologic malignances enables us to target angiogenic signaling pathways regulating tumor vascularity. Tumor-selectivity of angiogenic-targeting therapeutic approaches is expected to produce lower toxicity and higher efficacy than standard chemotherapeutic approaches. The functional contribution of lead angiogenic factors toward increased vascularity characterizing advanced metastatic tumors, must be considered in the context of the supporting vasculature and reactive stroma within the tumor microenvironment (Fig. 1). The fact that agents specifically inhibiting VEGF signaling have a limited efficacy in terms of tumor suppression translating into a meaningful therapeutic response, points to the realization that blocking VEGF signaling alone might not be sufficient to induce complete inhibition of tumor growth. Angiogenic factors controlling the dynamics of microenvironment with functional redundancy, are accountable for the resulting drug-resistance. Inhibition of VEGFR results in upregulation of PDGF and FGF-2 [Carmeliet, 2005; Casanovas et al., 2005]. Furthermore, one cannot underestimate targeting the supporting stroma-derived vasculature, since it may determine the metastatic potential controlled by PDGF [Blouw et al., 2003; Willett et al., 2005]. Combination

modalities may provide clinical benefit by enhancing endothelial cell sensitivity to anti-angiogenic/cytotoxic agents, in addition to delaying acquisition of drug resistance [Browder et al., 2000; Dong et al., 2004].

Overexpression of HIF correlates with the recurrence and progression of superficial uro-thelial bladder cancer [Klement et al., 2000], while in prostate tumors HIF expression correlated with expression of VEGF and androgen receptor levels [Theodoropoulos et al., 2005]. The recent screening of the small molecule inhibitor for HIF-1 activity [Boddy et al., 2005], may provide an effective lead in the development of novel approaches for optimizing existing angiogenesis-based therapeutic strategies in the treatment of urologic malignancies. Inorganic nanostructure "nanoparticle" technology has recently generated wide interest in the field of molecular medicine [Tan et al., 2005]. Nanoparticle has emerged as a novel intra-vascular probe for both diagnostic imaging (e.g., MRI; magnetic resonance imaging) and therapeutic intervention (drug delivery). Ruoslahti et al. recently developed a nanoparticle-tumor targeting strategy by securing delivery and stimulating its intravascular accumulation via "self-amplification," involving coupling clot-binding peptide with an MRI enhancer [Maynard and Pui, 2007]. Subsequent efforts established a novel protease-triggered self-assembly nanoparticle, "a neutravidin-and-biotin-functionalized superparamagnetic iron oxide nanoparticles," which amplify the assembly of nanoparticles, upon triggered by MMP-2 within the tumor site [Simberg et al., 2007]. This work provided new routes for exploiting tumor-delivered molecular therapeutics for the treatment of highly-vascular tumors without affecting normal vasculatity.

In summary, as the success of the therapeutic efficacy of the various anti-angiogenic approaches for the treatment of urologic tumors has yet to be proven in terms of long-term clinical benefit, the challenge remains to select targeting of key angiogenesis signaling pathways that can be tailor-made for and targeted to an individual tumor. The angiogenic profile of urologic tumors may vary depending on the age of individual patients and stage/grade of tumor [Harris et al., 2006]. Proteomic profiling of angiogenesis effectors dictating tumor vasculature in individual patients, will enable treatment optimization in order to bring about long-term control of urologic cancers.

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